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OBSERVATIONS ON A CASE OF SPLENOMEGALY WITH HYPERLEUKOCYTOSIS

NECROPSY FINDINGS SHOW THE PRESENCE OF MYELOGENOUS LEUKEMIA. ANALYSIS OF THE CASES IN LITERATURE*

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DURING the past few years there have appeared several reports of cases of splenomegaly with hyperleukocytosis particularly characterized by an increase of polymorphonuclear eosinophiles, or else mature neutrophiles. H. Z. Giffin[1] was the first to call attention to such a finding in a paper entitled "Persistent Eosinophilia with Hyperleukocytosis and Splenomegaly." His observation was made on a case of splenomegaly with a preoperative leukocytosis of 21,800 and a differential count exhibiting 73.6 eosinophiles. After splenectomy, the leukocytes quickly rose to 97,200 and later on to 211,000 with an eosinophilia varying from 79 to 90.7 per cent. At necropsy, which was performed 4½ years later, all the hemapoietic organs showed an abundance of eosinophilic myelocytes in the lymph glands and bone marrow.

Shortly after the appearance of this paper, an account was published by Tuohey[2] of a case where, following splenectomy, the patient's blood showed a very marked increase of leukocytes. No eosinophilic increase was present in this case but the augmentation was at the expense of polymorphonuclear neutrophiles. The conclusion of this author was that his case fell in the same category as the one described by Giffin. Aubertin and Giroux[3] then reported on a case of splenomegaly with persistent eosinophilia which, while differing in many ways from the case reported by Giffin, was considered by them to fall in the same group of affections. The most recent paper on this subject

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(at the time of this writing), is by McDonald and Shaw.[4] These authors reported a case of splenomegaly with eosinophilia, the clinical findings of which closely resemble those reported by Giffin.

Both Tuohey and Giffin were inclined to consider the cases under observation as manifestations of a hyperleukocytosis, rather than a true leukemia. McDonald and Shaw, on the other hand, are inclined to believe the case to be one of a leukemia of a special kind. Giroux and Aubertin consider the eosinophilia in their cases as due to anoxemia.

The literature on the subject also contains several other cases that are somewhat analogous to those under present consideration.

It shall be my purpose in this paper to analyze the reported cases and to make an attempt to conclude whether we are dealing with a newly observed clinical entity, or whether these cases fall into the category of previously described, and comparatively well-known diseases.

Before doing that, however, I shall report the clinical and pathologic findings of a case of splenomegaly which, while it presents certain features that make it quite unique in medical literature, will throw light on the cases we are now considering, and will serve to link them to well-known clinical entities.

CASE, A. C., male, aged fifty-two, shoemaker. Marked splenomegaly with normal (?) white and differential blood count. Splenectomy Dec. 26, 1914. Slow but good recovery. Four years later returned with clinical symptoms. Marked hyperleukocytosis found, with high percentage of eosinophiles. Liver markedly enlarged. Exacerbations and remissions. Died, 1921. Necropsy, myelogenous leukemia.

A. C., male, fifty years of age, born in Sweden, a shoemaker by occupation, entered the medical wards of the Waterbury Hospital on August 4, 1919. His family history was negative. His habits were good and he denied venereal infection. He had been married 30 years and had seven children, all living and well. The patient claimed to have been in perfect health until about seven years prior to admission. At that time, he noticed that he was easily fatigued, had some dyspnea on slight exertion, and was getting progressively anemic. He also noticed a sense of weight and discomfort in the left upper abdomen and soon afterwards observed that there was an abnormal swelling in that region. This mass caused a progressive and marked inconvenience, frequently amounting to actual pain, especially when sitting down and leaning over. In the recumbent position his distress would almost entirely disappear. He was examined by several physicians who told him that the mass in his left side was a large spleen and was advised to have the spleen removed. As the patient's anemia progressed and the discomfort from the enlarged spleen became more pronounced, he finally consented to an operation and had his spleen removed December 26, 1914, i.e., about two years after appearance of symptoms.

His convalescence was rather stormy and he remained in the hospital for nine weeks. He was in fairly good condition when discharged from the hospital and after a three months' rest, he returned to his work as a shoemaker and had enjoyed fair health. The patient apparently ate and slept well and seldom had any headaches but occasionally had dizzy spells. He managed to work 8 to 9 hours daily but any other form of exercise, particularly walking would soon fatigue him.

About five months prior to the present admission to the hospital, the patient commenced to feel worse; he grew tired very readily, began to have dyspnea on slight exertion; his pallor increased and all the symptoms that he had prior to his splenectomy, with the exception of the discomfort from the large spleen, returned with greater intensity. He also had frequent bleeding from gums and some cutaneous petechical hemorrhages.

His condition became progressively worse. He gave up working three weeks prior to admission and had been in bed for the last six days.

He was brought into the hospital (August 4, 1919) on a stretcher, and on admission, he presented an intense degree of pallor and an extreme state of weakness. He complained chiefly of dyspnea, constant thirst, palpitation, and a distressing, throbbing, sensation in the blood vessels of the neck and extremities. He had some cough and was expectorating some brown tenacious sputum tinged with blood.

Physical examination showed an extreme pallor and evidence of marked dyspnea.

The eyes reacted to light and accommodation. There was considerable pyorrhea of his gums which also showed evidence of recent bleeding. His neck showed evidence of both arterial and venous pulsations. There were many coarse dry and moist râles at both bases. His heart showed evidence of considerable enlargement. The apex beat was in the sixth interspace 4½ inches from the median line. On auscultation, a systolic murmur was heard best at the apex and in the fourth interspace to the left of the sternum. It

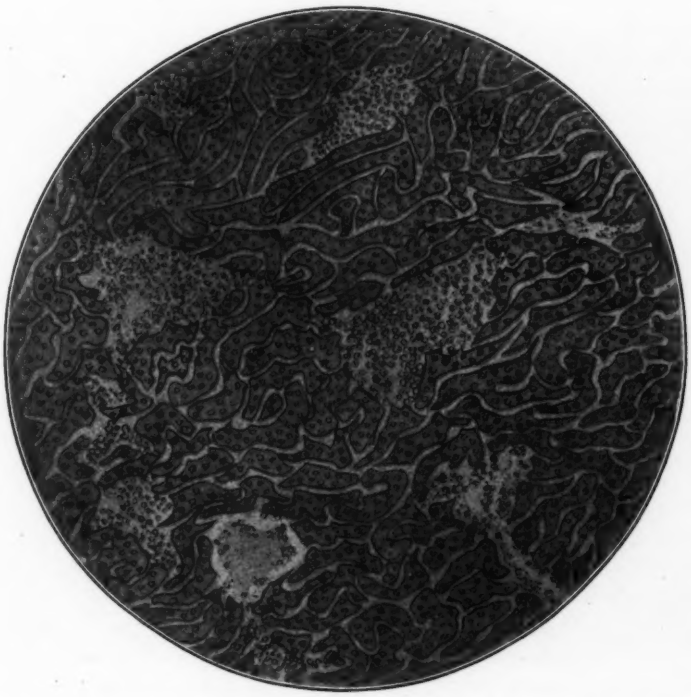

Fig. 1.—Section of liver showing marked leukocytic infiltration of organ.

was also heard over the entire precordium and in the axilla but it was not transmitted there by way of the apex.

The liver was markedly enlarged, its lower border being palpated 8 cm. below the costal margins. The outlines of this organ can be seen in the accompanying photograph. (Fig. 1.)

A rather extensive scar from the previous operation was present in the left hypochondrium. The scar tissue is stretched, appears quite thin in the middle and shows a marked pulsation which is synchronous with the systole of the heart.

There was no ascites, nor was there any dilatation or prominence of the superficial abdominal veins.

The cervical, axillary and other glands were not palpable.

There were no abnormal neurologic signs present. The nails of his fingers and toes showed a brown discoloration and marked atrophic changes, and he dated these changes to the time of the onset of his illness.

His temperature on admission was 97.4, pulse 82, respiration 22. His pulse was bounding and was suggestive of the Corrigan type. His blood pressure was 132/55.

Laboratory Findings: Urine: Light amber, acid 1014. Faint cloud of albumin, no sugar. Many hyaline and few granular casts. Many uric acid crystals.

Blood: Hemoglobin 18 per cent (Sahli); R.B.C. 1,240,000; W.B.C. 150,200; Polys, 59.5 per cent; Lymphs, 11 per cent; Trans., 3 per cent; Eos., 24.5 per cent; Bas., 0; Myelo., 2 per cent. There was an extraordinary number of nucleated red blood cells, equaling 37.5 per cent of the leucocytes as seen in smears. (Total 56, 325 per c.mm.) The patient's bleeding time was seven and coagulation time was five minutes. The Wassermann test was negative. The Blood Chemistry showed a Blood Urea of 38 mg. per 100 c.c.; Uric acid 3.5 mg.; Sugar .09 per cent and Creatinine 1.2 mg. per 100 c.c. of blood.

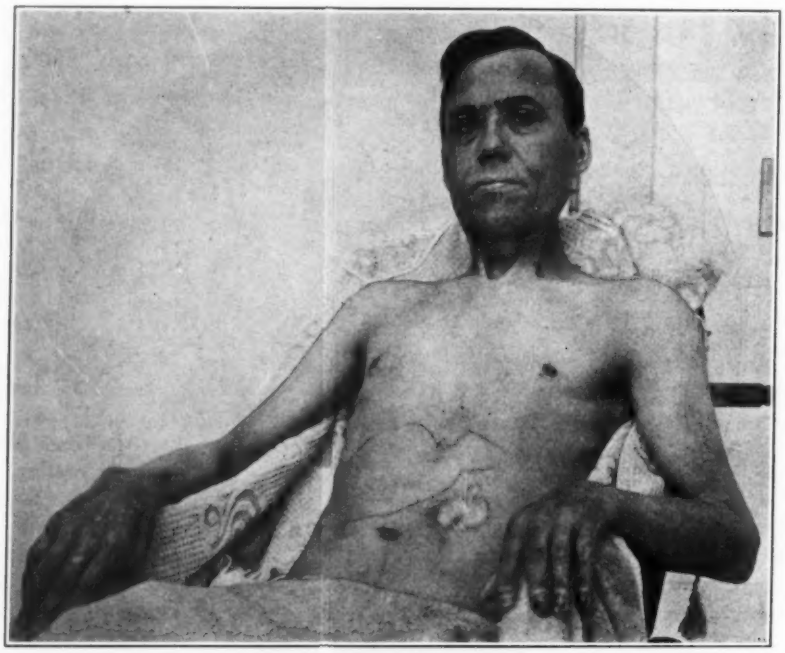

Fig. 2.—Photograph of patient (A.C.) showing general appearance and degree of enlargement of liver below costal margin.

The patient's condition on admission to the hospital was extremely poor. A transfusion was performed the day after admission and the patient received 600 c.c. of blood by the citrate method. The results from this procedure were very pronounced and the patient promptly began to improve both in color and in strength.

SUBSEQUENT INVESTIGATIONS

The records of the patient's stay in the hospital in 1914 when he had splenectomy performed, were, unfortunately, lacking in a great many points that are extremely important, especially in the light of the extraordinary blood changes that were encountered at this time. The only blood count found was a white and differential done prior to the operation. This was

recorded as W.B.C. 9,400 and a differential count of Polys 72 per cent and Lymphocytes 38 per cent. No other records are present that would have any bearing on the case.

Fortunately, the spleen was preserved, and although it was considerably deteriorated during the time elapsed, it was still of great aid in the subsequent study of the case. The organ weighed 2200 gm. in the hardened state. The normal shape of the organ was well preserved. The surface was smooth,

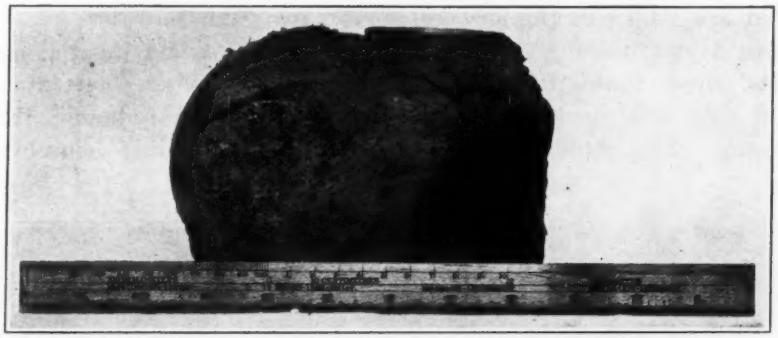

Fig. 3.—Section through spleen showing general appearance and size.

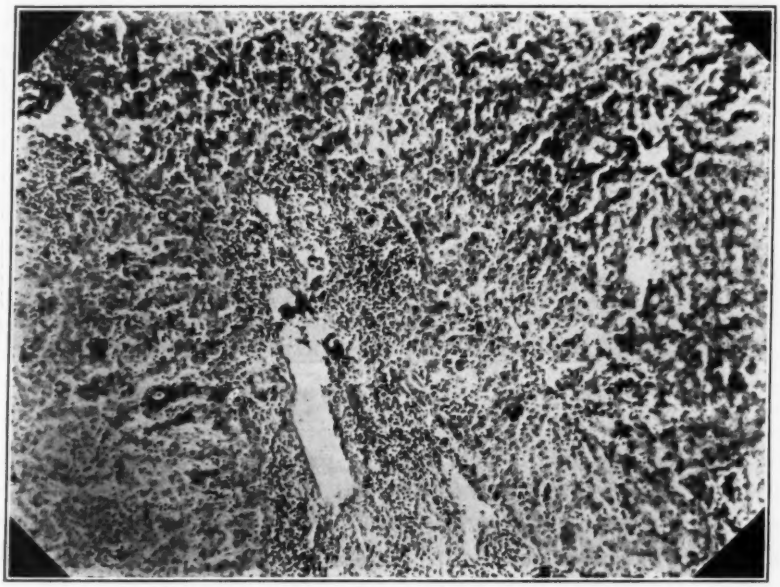

Fig. 4.—Microphotograph through liver showing marked degeneration and cellular infiltration. B. and L. Obj. 2/3. Oc. 1.

white and coated with grayish-white areas of fibrous tissue, apparently due to perisplenitis. On gross section, the organ presented a firm, homogeneous appearance. The malpighian follicles were not visible.

Microscopic examination of the organ showed that the enlargement was due to a cellular infiltration and a proliferation of fibrous connective tissue. The cells that infiltrated the organ were mostly polynuclear in character.

They contained neutrophilic and eosinophilic granules. Myelocytes were but rarely seen. A number of mononuclear, nongranular cells were also seen. Also, occasional macrophages were encountered. These contain masses of pigment, also leukocytes and fragments of nuclei. As was said above, the fibrous tissue was greatly increased and many bands were seen running along the sinuses and capillaries. In some areas the fibrous tissue predominates the histologic picture.

The details of the patient's blood examination and the fluctuations it presented are shown in the accompanying table and chart.

Three days after admission to the hospital, the patient was put on benzol (15 mins. three times daily). From the accompanying chart it may be gathered that this drug was instrumental in greatly reducing the white blood count. This does not seem to be at all likely since benzol adminis-

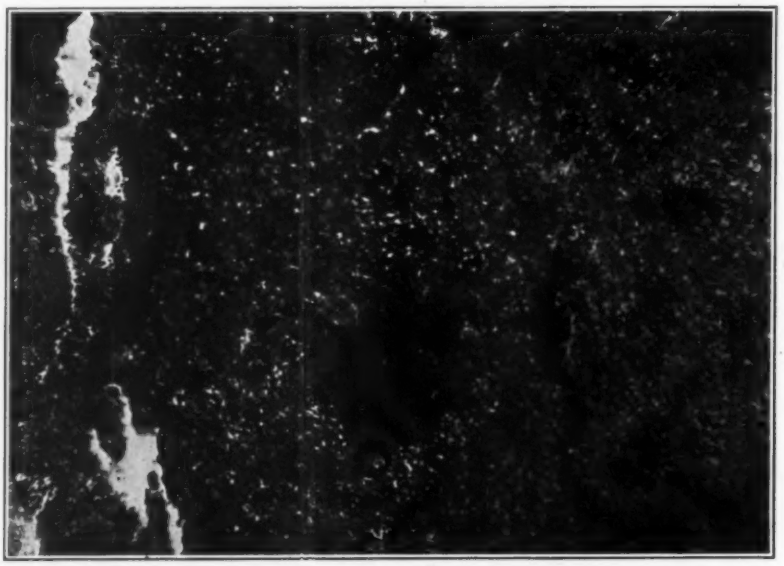

Fig. 5.—Microscopic section through spleen. (Low power.)

tered in the same dosages later on in the progress of the disease (December and January) had no noticeable effect on the number of white blood cells. The first diminution of the cells seemed to be due to the transfusion and to the hematinic medication (iron and arsenic) that the patient received in the hospital. As will be noticed from the table, arsphenamine intravenously had no appreciable effect in diminishing the number of leukocytes.

During the twenty-three months that the patient remained under observation, the white blood count varied from 16,000 to 70,000; most frequently between 30,000 and 50,000. The hemoglobin and red cells also varied greatly although to a lesser extent.

The patient's general condition presented fluctuations which were not always dependent on the changes in the blood. They were most frequently due to some intercurrent complication. Thus for example, in July, 1920,

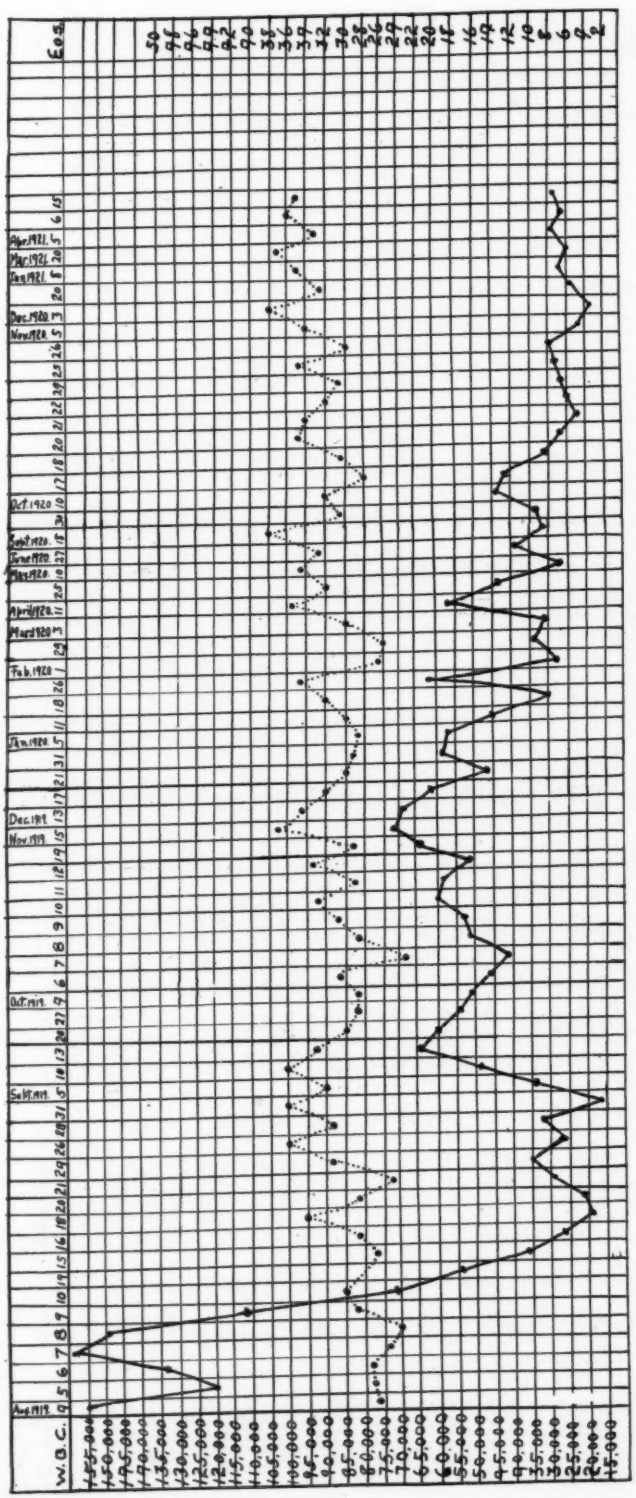

Chart 1.—(A.C.) Curves indicating white blood count and eosinophilic count.
W.B.C. ————
Eosinophiles .....

TABLE I

A.C. TABULATION OF COMPLETE BLOOD COUNTS TAKEN DURING PERIOD OF OBSERVATION

DATE	HB.	R. B. C.	W. B. C.	POLYS	LYMPHS	MON.	TRANS.	EOS.	BAS.	MYELOCYTES			
										N.	E.	B.	TOTAL
8- 4-19	18	1,240,000	150,200	59.5	11	0	3	24.5	0	2	0	0	2
8- 5-19	18	1,660,000	121,300	56	15	0	3	25	0	1	0	0	1
8- 6-19			130,600	64	7	0	2	25	0	1	1	0	2
8- 7-19	20	1,210,000	160,000										
8- 8-19	35	2,040,000	148,000	50	19	2	3	22	0	2	1.5	.5	4
8-10-19	36	2,010,000	112,000	64	4	3	0	27	0	1	1	0	2
8-14-19			73,200	59	9	0	2	28	1	0	0	1	1
8-15-19			54,300										
8-16-19			36,700	50	15	2	3	25	3	1	1	0	2
8-18-19			27,300	48	17.5	2	1.5	26.5	1.5	2	1	0	3
8-20-19	42	2,310,000	19,200	44	17	1	1	32	2	2	1	0	3
8-21-19			21,600	51	14	2.5	.5	26.5	3	1.5	0	1	2.5
8-24-19			28,200	60	8	3	3	23	1	1	1	0	2
8-26-19			34,600	43	4	5	0	29.5	6	2	1	.5	3.5
8-28-19	38	3,170,000	27,600	48	9	2	1	34	4	2	0	0	2
8-31-19			31,700	52	12	0	.5	29	3.5	2	1	0	3
9- 5-19			16,800	43.5	16.5	1	.5	34	3.5	0	0	1	1
9-10-19	45		34,800	52	12	0	1	30	3	1	1	0	2
9-13-19			54,400	44	16	1	1	34	2	1	1	0	2
9-20-19	45	2,100,000	63,600	45	18	4	3	31	1	5	[illegible]	1	3
9-27-19	38	2,050,000	60,000	50	16	2	1	28	1	1	1	0	2
10- 4-19	36	2,100,000	54,200	52	15	1	1	27	2	1	0	1	2
10- 6-19			52,100	49	21	2	1	27	1	2	0	0	2
10- 7-19			46,800	53	8.5	3.5	3.5	28.5	1.5	1	0	.5	1.5
10- 8-19			47,200	56	15	4	2.5	21.5	0	0	1	0	1
10- 9-19	35	2,500,000	52,800	55	12	1	1	27	2	1	1	0	2
10-10-19			53,600	46	15.5	1.5	3.5	30	2.5	1	0	0	1
10-11-19			60,000	51	15	2	2	27	2	0	1	0	1
10-12-19			58,300	53	11	3	2	31	0	0	0	0	0
10-14-19			52,200	55	0	5	3	27	0	1	0	0	1
11-19-19	42	2,600,000	64,000	49	7.5	6	2	35	.5	0	0	0	0
12-13-19	60	2,200,000	72,000	49.5	3	7.5	2.5	32.5	3	2	0	0	2

TABLE I—CONT'D

A.C. TABULATION OF COMPLETE BLOOD COUNTS TAKEN DURING PERIOD OF OBSERVATION

DATE	HB.	R. B. C.	W. B. C.	POLYS	LYMPHS	MON.	TRANS.	EOS.	BAS.	MYELOCYTES			
										N.	E.	B.	TOTAL
12-17-19	65	2,760,000	69,000	40	16	8	3	30	1	0	2	0	2
12-21-19	60	2,000,000	62,400	48	18	2	1	28	1	1	1	0	2
12-31-19	65	2,920,000	48,000	50	14.5	3	2	27.5	1.5	1	.5	0	1.5
1- 5-20	70	2 840,000	59,200	51	12	7	2	27	0	1	0	0	1
1-11-20	72	2,980,000	58,600	54	12	4	0	28	1	0	1	0	2
1-18-20			45,400	59	7	2	0	30	0	1	0	1	1
1-25-20			30,000	54	6	4	2	33	0	1	0	0	2
2- 1-20	70	2,800,000	62,000	61	7	4	1	25	0	1	1	0	1
2-29-20	75	3,660,000	28,800	53	10	7	3	24	2	1	0	0	1
3- 3-20			34,000	55	9	4	2	28	1	0	1	0	2
4-11-20	85	4,370,000	32,600	52	3.5	5	3	33.5	1	2	0	0	
4-25-20	70	3,270,000	56,700	59	5	4.5	5	30	0	0	0	0	1
5-10-20	72		44,200	56	4	3	1	33	1	1	1	1	2
6 27-20	82	4,170 000	28,200	57	4	2	3.5	31.5	1	1	0	0	1
8-18-20	70	4,100,000	38,200	50	8	4	0	36	1	1	0	0	1
9-30-20	70	3,120,000	31,000	55	12.5	0	2.5	28.5	1.5	0	0	0	0
10-10-20	70		32 000	62	6	2	0	30	0	0	0	0	0
10-17-20	72	3,670,000	43,800	65	6	1	2	26	0	0	0	0	0
10-18-20			42,600	54	13	1	1	29	0	1	0	1	2
10-20-20			31,800	58	6	1	0	33	1	0	0	1	1
10-21-20			27,500	55	10	0	1	32	2	0	0	0	0
10-22-20	68	3,400,000	22,100	55	13	0	1	30	1	0	0	0	0
10-24-20			25,000	54	8	5	0	29	3	1	0	0	1
10-25-20			25,600	53	8	2	0	33	3	0	1	0	1
10-26-20			27,400	60	8	2	0	28	1	1	0	0	1
11- 5-20	64	3,400,000	28,200	48	6	0	2	32	1	1	0	0	1
12- 3-20	62		22 100	40	14	4	2	36	2	0	2	0	2
12-20-20	64		18,600	41	12	8.5	3.5	31	3	0	1	0	1
1- 8-21	58	2,900,000	24,200	39	12	9	4	33	2	0	1	0	1
3-20-21	60		26,100	55	4	3	1	35	2	0	0	0	0
4- 5-21	46		25,600	51	8	4	3	31	2	0	1	0	1
4 -6-21	40	2,100,000	28,300	54	4	2	1	34	3	1	1	0	2
4-15-21	32	1,600,000	32,400	57	4	1	2	33	1	0	1	1	2

the patient had arthritis of the right wrist. It was accompanied by redness and swelling, but no constitutional symptoms. Temperature was normal. In August, 1920, the patient had a tenosynovitis of the forearm. All through the summer of 1920 the patient had recurrent bleeding from the gums.

The various infections that the patient had while under observation produced no appreciable changes in the blood.

On April 13, 1921, the patient was readmitted to the Waterbury Hospital, suffering from bronchopneumonia. The blood findings at this time were about the same as observed during the preceding months.

The patient's condition grew progressively worse and he died four days after admission to the hospital.

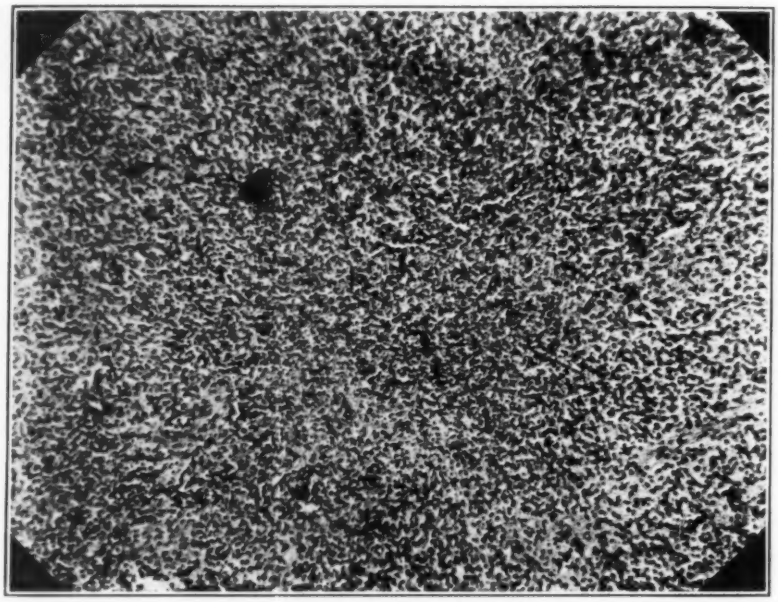

Fig. 6.—Section through accessory spleen showing increase of connective tissue and cellular proliferation.

Necropsy

A. C. 4-17-21.

Skin of icteroid appearance.

Abdominal organs:

Liver: Very large and yellowish brown in color. It is firmly adherent to the surrounding structure. In the gross section it was found to be yellowish and firmly smooth.

Gall Bladder: Large, full of bile, contains no stones.

Splenic pocket: Clean, a few adhesions in the splenic region.

Intestines somewhat distended, colon adherent to the peritoneum. Descending colon and sigmoid are full of hard masses and fecal material.

Duodenum: Small and superficial, also thickened. Several small ulcers with hemorrhage found in first portion. The largest of these is ½ cm. in diameter.

Appendix: Club shaped, bent on itself and is retrocecal.

Pancreas: Apparently normal and weighed 120 gm.

Mesenteric Glands not enlarged.

Adrenals not enlarged. Right icteroid: on section shows white thickening around central artery. Left presents same picture as the right.

Thorax: Opened from the sixth rib down, 13 cm. from midsternum. The ribs are seen to be pressed together on each side. Well marked bronchopneumonia and edema in upper lobe of left lung.

Prevertebral tissues yellowish and edematous. Thoracic duct distended. A retro-esophageal gland found enlarged and was removed for sectioning.

Kidneys: Right weighed 180 gm. Capsule stripped readily. Smooth surface, but a little granular in places. Is somewhat enlarged. On section cortex is found swollen and pale yellowish. Medullary parts edematous. The left kidney is similar to the right kidney, weight, 200 gm. Over the anterior surface of the kidney, a small hard mass, the size of a split pea is present. This was removed for sectioning.

Prostate small and firm.

Left Femur: Periosteum shows no evidence of disease, marrow cavity found to have a yellowish tinge.

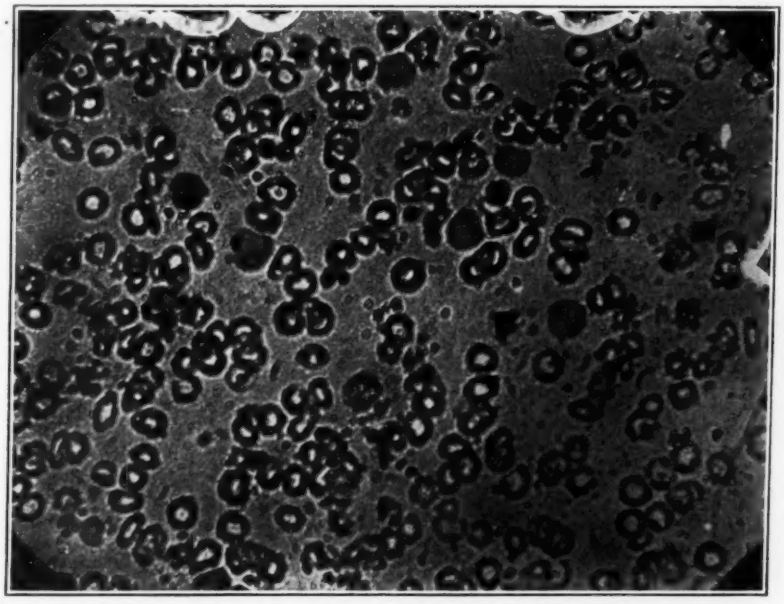

Fig. 7.—Microphotograph of blood (X750) showing increase of leukocytes and the presence of nucleated reds.

Heart: Much enlarged and covered with fat. Shows evidence of old pericarditis over right auricle. Weight, 700 gm. The mitral valve admits two fingers. There is a large fat clot in the right auricle. Tricuspid valve admits two fingers. Aorta shows several small plaques. The coronary vessels appeared normal.

Area of papillary muscles completely converted into mottled fatty tissue but contains small greenish blue gray spot resembling tissue found in the adrenals on section.

Lungs: Left lung very adherent, weight—8800 gm. Right lung not adherent. Weight —1050 gm. Glands around hilus of lung small. Lower near the vertebral column feels firm. Fibrinous pleurisy. Small thrombi seen in blood vessels on section.

Morbid Histology

The Lymph Glands: Sections taken from the peribronchial, retroperitoneal and cervical glands, all showed a similar structure. The lymphoid tissue was well developed. The germinal centers were as a rule, absent. Many areas were present where the lymphoid tissues had been replaced by a dense accumulation of polynuclear cells. On higher power

examination, the cells were found to be either neutrophiles or eosinophiles. Myelocytes were present in very small numbers.

The Bone Marrow: Smears of the bone marrow, stained with hematoxylin and eosin and by Wright's stain, showed numerous cells of the polynuclear type. The majority of these cells were eosinophiles in character. About 8% of the cells were mononuclear with distinct neutrophilic and eosinophilic granulations.

A moderate number of red blood cells were found and about 70% of these were nucleated.

A number of myelocytes were found in the process of division.

Sections through the bone marrow showed similar findings to that obtained from the smears.

The parenchymatous cells of the *liver* were in an advanced state of cloudy swelling. Glisson's capsule was found infiltrated, whilst the interlobular veins were found degenerated and in places replaced by masses of polynuclear cells lying between the liver cells. The capillaries were stuffed with leukocytes similar to those found in the bone marrow and lymph glands. Considerable amount of iron pigment was observed.

The Lungs: The sections taken from the small bronchioles and from the parenchyma of the organ showed findings similar to that of the liver but to a lesser extent.

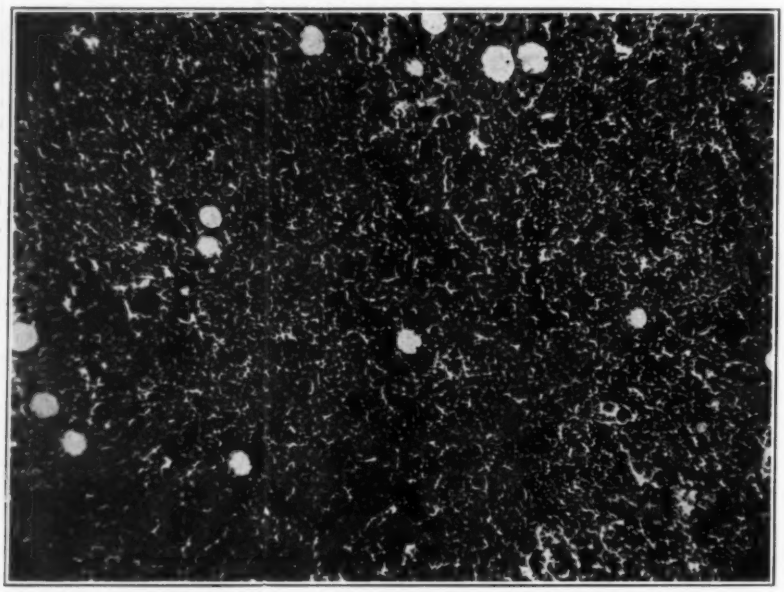

Fig. 8.—Microphotograph of bone marrow taken from the tibia.

The histologic sections of the various organs were submitted to Dr. Douglas Symmers, Professor of Pathology at University and Bellevue Hospital Medical College. In a personal communication to the author, Dr. Symmers expressed his opinion that the case is undoubtedly one of leukemia. He found evidence of it in all the organs submitted. To quote from his communication, "I do not think there is any doubt but that you are dealing with a case of leukemia. * * * In the accessory spleen, I see no reason to make a diagnosis of Banti's disease and in this connection it is to be recalled that accessory spleens always present identical changes with the main spleen. The spleen taken out five years before death is poorly preserved and I cannot make much out of it that is of value. It is therefore impossible for

me to say whether the patient was leukemic at the time of splenectomy or whether it developed later."

DISCUSSION

From the clinical standpoint the case deserved the following considerations which presented themselves for differential diagnosis:

1. Hyperleukocytosis following splenectomy.
2. Banti's disease with superimposed leukemia.
3. Leukemia.
4. Myelogenous leukemia.
5. Splenomegaly with eosinophilia of special type.

1. Hyperleukocytosis following splenectomy. As will be noted from the above records and tables, the patient's white blood count came down to 19,000 within three weeks after the blood transfusion and had not risen to a much higher figure for a long time afterward. With these findings, especially in view of the almost complete absence of myelocytes in the blood smears and the history of Banti's disease which the patient gave, one could easily consider the case as one of a persistent and unusually high hyperleukocytosis following splenectomy. This consideration deserves some analysis and comment.

The subject of hyperleukocytosis following splenectomy has been studied quite exhaustively, both clinically and experimentally. Of the early observers, we may mention Vulpius[5] who found a leukocytosis of 21,000 to 26,000 during the first 40 days following splenectomy. After that time the leukocytes gradually diminished and reached the normal level at the end of about 65 days. Gibson[6] experimenting on the dog, found a leukocytosis of 14,000 to 17,000 lasting about 15 months. DaCosta[7] gives a leukocytosis of polyneutrophilic type of 15,000-30,000 persisting for 4 to 5 weeks.

While there are a great many records of blood counts following splenectomy in man to be found in the literature on the subject, it is evident that most of the cases in this class cannot be taken as indications of changes due to the splenectomy since the operation is almost invariably performed because of disease changes which in themselves are capable of altering the blood picture. There are, however, several cases of splenectomy in man on record which, because of the circumstances of the cases, may be accepted as indicating blood changes due solely to the splenectomy.

Henricius[8] found in a case of a cyst of the spleen, with a normal preoperative blood count, a postoperative leukocytosis of 19,800. Five months later, there was a leukocytosis of 13,200 and 17 months later, it was 12,000. Warren[9] reports a case of splenic anemia, where the preoperative white blood count was 2,200. On the sixteenth day following operation, there was a leukocytosis of 24,000. Hartmann and Vaquez[10] reported a case with a postoperative leukocytosis of 17,000. One year later, the leukocytes numbered 27,000. Musser,[11] reports a case of a cyst of the spleen. Eight days after splenectomy the leukocytes numbered 11,400. Fifteen months later they numbered 14,800 per cm. Eliot[12] in a case of splenectomy for rupture of the spleen, found one year after operation a leukocytosis of 17,000. Meyers[13]

found a postoperative leukocytosis of 8,400 and two months after operation, it was 14,100.

Among the most recent contributions to this subject are the observations made by Hall.[14] This author reports the results of careful blood examinations made for a period of three months in a case of splenectomy which was performed for a slight rupture with moderate hemorrhage. The increase of the leukocytes was irregular, never rose very high and presented relative increase of the endothelial cells.

The above, carefully selected investigations in man will serve to indicate that a leukocytosis generally takes place after splenectomy but it is usually moderate in extent and does not seem to last more than several months, or a year or two at the most.

Experimentally, similar changes were noted. Vulpius (above citation), found the postoperative leukocytosis to subside at the end of 65 days. Pierce *et al*[15] gives a postoperative leukocytosis in dogs ranging from 14,000 to 38,000 with a gradual decrease and reaching the normal in one to four months. Similarly, Johnstone[16] in the course of investigations on changes in morphology and function of bone marrow, found after splenectomy that the leukocytic increase oftentimes occurs almost immediately after splenectomy and may be very striking, but is quite temporary and inconstant.

So far as the eosinophilic cells are concerned, there are no records in the literature on the subject of any striking increase of these cells following splenectomy. DaCosta (as mentioned above) states in his textbook that an eosinophilia may develop after some months, or a year or two after splenectomy, but most other observers have not noticed it. Hall[17] for instance, gives careful blood findings in a case of splenectomy extending over a period of over three months, but has not been able to find an increase of the eosinophiles.

It will become quite apparent, therefore, that it is extremely unlikely that such striking and persistent blood changes as presented by the case we are describing should have been produced by the removal of the spleen; and it is absolutely safe to exclude that from our differential diagnosis.

BANTI'S DISEASE WITH SUPERIMPOSED LEUKEMIA

The consideration of this possibility deserved some attention by virtue of the normal white blood count at the time of operation and the clinical diagnosis of Banti's disease that was made at that time.

However, when we consider that the literature on the subject (as far as I have been able to ascertain) has no record of any case of leukemia following splenectomy in Banti's disease, nor superimposed upon a case of this symptom complex. Also, that many exceedingly competent investigators completely deny its presence as a disease entity. While the considerable deterioration of the spleen in this case prevented the study of the histologic details to the extent which it deserved, it has nevertheless shown a sufficient amount of structural details to throw it out of the category of Banti's disease. The cellular hyperplasia speaks decidedly against it; similarly the absence of increase of connective tissue as demonstrated not only by the ordinary methods but by the Van Gieson and other connective tissue stains.

The fact that the patient survived the splenectomy and the comparatively long duration of life in no way disprove the possibility that the patient had a chronic, low grade leukemia at the time the operation was performed.

While one ordinarily associates an exceedingly high mortality rate in splenectomy for leukemia, there are cases on record where the patients made good recoveries and lived for several years after the operation.

Neither does the normal blood count at the time of operation rule out the presence of myelogenous leukemia. Many competent observers admit that in the course of this disease one may encounter periods where the white blood count is quite normal. As authority for this statement I may quote, among others, Emerson[18] and Cabot.[19] Giffin[20] reports a case of splenectomy whose leukocyte count prior to operation was 11,000 and whose differential count showed no myelocytes. Two years after operation, however, the W. B. C. was 14,000 with 14 per cent myelocytes. I shall have occasion to refer again to this case later on; but for the present, it will serve to add to the evidence that there certainly are periods in the course of myelogenous leukemia where the W. B. C. may appear quite normal.

Therefore, while it is conceivable that a patient with Banti's disease may get a superimposed leukemia, the histology of the spleen and the subsequent findings in the case safely rule it out.

LEUKANEMIA

The comparatively high color index that the patient presented when first seen, the extreme anemia and the high leukocytosis brought to one's mind the symptom-complex that has been called leukanemia. But the consideration of this falsely defined symptom-complex can be easily discarded. Although when first described by Von Leube[21] and later by Luce[22] it was temporarily accepted that a condition exists where the patient suffered from both pernicious anemia and leukemia, it soon became apparent that no such disease entity occurs. Critical analysis of the reported cases showed that they may easily be relegated to either one or the other of the two diseases. As a rule they are atypical cases of myelogenous leukemia. While textbooks and monographs on diseases of the blood still mention leukanemia, it is as a rule, only to condemn the employment of the term and to deny its possibility.

REVIEW OF CASES IN LITERATURE

I shall now proceed to briefly analyze the cases reported in the literature as unusual forms of splenomegaly with hyperleukocytosis.

The case reported by Giffin is summarized by him as follows:

DISCUSSION

F. H., male, aged thirty-one years. Marked splenomegaly. Slight enlargement of superficial lymph glands. Leukocytosis 21,800 before splenectomy with an eosinophilia of 73.6%. Splenectomy July 15, 1914. Weight of spleen 2,110 gm. Macroscopic appearances of spleen similar to that of myelogenous leukemia. Rapid increase of leukocyte count to 97,200 and later to 211,000; eosinophilia from 79 to 90.7%. Good general condition for period of four years following splenectomy. Death January 19, 1919, of empyema

following pneumonia. Necropsy: Enormous numbers of eosinophilic polymorphonuclears in all hemopoietic organs. Eosinophilic myelocytes numerous in lymph glands, spleen, and bone marrow. Obliterative pericarditis and fibrous perihepatitis.

It will be noticed from the report that the macroscopic appearance of the spleen was "similar to that of myelogenous leukemia." At autopsy eosinophilic myelocytes were found in great number in lymph glands, spleen, and bone marrow. It does not mention any search for them in any other organs.

The clinical picture and the postmortem findings rather than disproving the presence of a myelogenous leukemia, actually render it highly suggestive of it. The atypical blood picture may be said to be quite characteristic of the more chronic forms of leukemia and are encountered rather frequently in the more protracted cases. To illustrate this point, I can cite one of the cases reported in another paper by the same author[23]:—

CASE No. 27,414. Case of a man, aged forty-five, whose leukocyte count was 11,000 and whose differential count showed no myelocytes. The spleen was removed in August 1909. Two years later the leukocytes were 64,500 with 14 per cent myelocytes. The patient died Dec. 27, 1914, five years and four months after the operation, with the symptoms of cardiorenal insufficiency. The spleen in this case weighed 2500 gm. This was evidently a leukemia of a more chronic type, as splenomegaly had been present four years preceding the operation. The total length of the history was, therefore, at least nine and one-half years.

Tuohey's case[24] may be summarized as follows:

Female, aged fifty-eight, with negative past history, except that she had had acute arthritis when 16 years of age. Her present illness started with pain in her side, and on examination, she was found to have an enlarged spleen. On physical examination nothing was discovered beyond a pessary, which she had worn for 14 years. There was local irritation as a result; this soon subsided. A blood examination showed a leukocytosis of 65,000. A differential count showed 99 per cent polymorphs. The discomfort and abdominal distress suggested the advisability of splenectomy. This was carried out successfully with an uninterrupted recovery. The leukocyte count rose, however, in three days to 240,000 but the leukocytosis subsided in about a month to 45,000. For over a year she was able to carry on her household duties, but some seventeen months after she succumbed to an acute pneumonia after a week's illness. Unfortunately no details of the blood condition after she left the hospital in 1917 are available, and an autopsy was not permitted.

This case is probably one of leukemia, but since no autopsy was performed and the spleen was not reported upon, it seems unwise to make any serious attempt at classification. The resemblance to the case of Giffin is only superficial. It only resembles it insofar as it presents a splenomegaly with a hyperleukocytosis, the differential count of which is not characteristic of myelogenous leukemia. More than this, we cannot say.

Aubertin and Giroux report a case which may be summarized as follows:

The patient was under observation for four years suffering from dyspnea and cyanosis. A diagnosis of primary sclerosis of the pulmonary artery with consecutive cardiac insufficiency was made. The spleen was slightly enlarged. There was a marked permanent eosinophilia; the total white cell count averaged about 10,000 per cmm., varying between 6,900 and 26,000. The percentage of eosinophiles remained constantly between 65 and 70.

A parasitic origin of the eosinophilia was excluded; the Wassermann reaction was negative. The patient died of cardiac failure. The autopsy confirmed the clinical diagnosis of sclerosis of the pulmonary artery but, unfortunately, no study of the blood forming organs was made.

The authors consider this case as similar to that of Giffin, especially since both had serious heart disease and advance the theory that the eosinophilia was due to anoxemia. Since the spleen was but slightly enlarged and the W. B. C. never rose above 26,000, it seems very unlikely that it should belong to the group of leukemias.

The case reported by McDonald and Shaw[25] is that of a male, aged 46, with a negative family and past history. The patient came under observation in July, 1921. His chief complaint was loss of weight and strength. On physical examination the patient showed the presence of a large spleen. The glands were not palpable. The blood examination showed a leuocytosis of 34,000 per cmm. and the differential count showed 71.4 per cent eosinophiles. A slight degree of secondary anemia was present.

This case is most likely one of myelogenous leukemia and the further reports of the authors on this case will be of extreme interest.

The spleen was removed fifteen days after admission to the hospital and the patient made an uninterrupted recovery. Since the operation, the leukocytes constantly increased in number, rising in December, 1921, to 92,800 per cmm.; the percentage of eosinophiles has remained fairly constant and has risen now to 82 per cent.

The removed spleen weighed 1276 gms. and measured 18.5 × 14.5 × 8 cm. On section the pulp was uniform in appearance but grayish pink in color, the malpighian bodies could be seen, but were not prominent. The histology of the organ (also that of two lymph glands found near the hilus) is given in great detail. It showed a great accumulation of eosinophile cells, and polymorphonuclear leukocytes were found in small numbers. An occasional giant cell with multilobulated nucleus was also observed in the pulp of the organ.

The hemolymph glands also showed the presence of numerous eosinophilic cells, both leukocytes and myelocytes.

This patient remained under the observation of the authors who reported the case. In September, 1922, his general condition was found to be failing and his blood examination showed W.B.C. 138,250 per cmm. The differential count showed 79.3 eosinophiles.

SUMMARY AND CONCLUSIONS

1. A case is reported that resembled the cases of "Splenomegaly with Hyperleukocytosis" that appeared in the literature within the past few years.

2. A detailed postmortem study of this case showed it to be an atypical form of myelogenous leukemia.

3. An analysis of the cases of "Splenomegaly with Hyperleukocytosis" in the literature, leads the author to believe that there is no justification from

the findings of the reported cases to consider them as a new clinical entity, which has not been described previously.

4. The cases to which we are now referring are atypical forms of myelogenous leukemia. A few of the cases in this group cannot be classified because of insufficient clinical and pathologic data.

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THE ACTION OF QUINIDINE ON THE AMPHIBIAN HEART*

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SINCE the work of von Frey[1] about five years ago, there has grown up a voluminous literature on the cardiac action of quinidine. This is chiefly clinical, though there is no paucity of the experimental. In both the attempt has been made to determine the exact mechanism of action on the heart and to localize it in some specific anatomical constituent of the cardiac complex. While there is general unanimity as to its clinical effect in allaying fibrillation of the auricle, there has been lack of agreement as to its *modus operandi*. The earliest investigators concluded that it was essentially a muscle poison (Santesson[2]); while the most recent students locate its chief points of action in the Purkinje fibers, the branches of the conducting system, and the terminations of the vagus nerve (Lewis *et al*[3] and Korns[4]).

The purpose of this paper is to present a study of the effects of quinidine on the reptilian heart. In carrying out our experiments, we have adopted freely the methods and procedures of other investigators of cardiac drugs, frequently, however, introducing original technic and devising special apparatus. We have endeavored to analyze the important reactions through a critical study of the changes produced in the several elements of the cardiac apparatus on which they depend. Each observation was repeatedly controlled in a series of 150 experiments; but, for the sake of brevity, we have here summarized the important effects rather than cited in detail the data from the individual animals.

METHODS AND MATERIALS

The animals employed were turtles and frogs. Since both reacted qualitatively alike, the first mentioned were found more practical on account of the greater size of the heart. The frogs were used chiefly for confirmation of observations.

Most of our experiments were made during the cool days of fall and spring. The turtles were kept out of doors in a pen, where they had free access to a large shallow pond partly covered with flagstones. They were fed chiefly on beef scraps and loaf bread, though occasionally on the carcasses of frogs and rabbits. In the cooler weather, they were put in the laboratory at least twelve hours before being used. The frogs were kept in a tank in the basement of the building. Since each batch was used up within a few days, they were not fed.

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The hearts of these animals were examined at room temperature (1) *in situ*, (2) perfused, and (3) immersed as follows:—

1. The frogs were anesthetized with urethane or pithed cerebrally. The turtles were always pithed. Their hearts were exposed in the usual way, care being exercised to lose little blood. Simultaneous tracings of the auricle and ventricle were made with levers attached by fine ligatures to the apices.

2. The perfusion experiments were made only with the hearts of turtles. These were quickly removed and placed in an operating pan containing Ringer's fluid. Where the whole heart was to be examined, cannulas were inserted in the vena cava and aorta, the perfusate entering the former. Auriculoventricular tracings were taken as in the case of those *in situ*. Excised auricles were perfused from the vena cava through the auriculoventricular opening and excised ventricles from the latter through the aorta. Ringer's fluid, through which oxygen was kept bubbling, was used in all cases. The inflow was maintained under a pressure head of 5 cm. and the outflow 7.5 cm., so that the internal pressure of the fluid was 2.5 cm. It was kept constant at the above level by means of a simple little valve placed

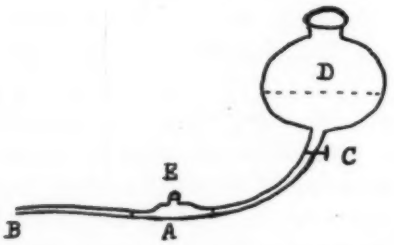


Fig. 1.—A diagram showing the arrangement of the constant pressure valve. *A*, The valve, consisting of a T-tube with the horizontal portion expanded; *E*, opening through which fluid is kept "wasting," so that *E* is maintained as the pressure head; *B*, to vena cava; *C*, stop-cock; *D*, reservoir.

between the reservoir and the heart. By means of it the perfusion pressure, or internal pressure, could be held constant to within one to three mm. of water, a variation which experience showed to be negligible. The arrangement of the valve is best explained by reference to Fig. 1.

3. Immersed. The apparatus and procedure were like those employed in experiments on the excised intestine and uterus. Whole hearts of frogs and turtles, and strips from the auricles and ventricles of turtles, quickly prepared, were suspended in oxygenated Ringer's fluid.

The sulphate of quinidine was used throughout, since it is the salt commonly employed in clinical work. It was administered in the *in situ* experiments subcutaneously, intravenously, superficially, and intrahepatically (5); in the perfused, in dilute solution in Ringer's fluid; and in the case of the immersion preparations by adding a solution of the drug until the bath reached the desired concentration.

The effects were not nicely quantitative. Application to the surface of the exposed heart (and even injection into the pericardial sac) produced reaction only tardily and did not permit of a determination of dosage. Subcutaneous administration was hardly better, there being evidence of im-

perfect absorption. Only the intrahepatic, the perfusion, and the immersion methods gave approximately uniform results. In connection with these the dosage employed and the designations used in the later parts of this paper are as follows:—

TABLE I

THE DOSAGE EMPLOYED IN MOST OF OUR EXPERIMENTS

DOSAGE	METHOD OF ADMINISTERING		
	INTRAHEPATIC	PERFUSION	IMMERSION
Small	1 mg.	1:20000	1:3000
Intermediate	2 mg.	1:5000	1:2000
Massive	4 mg.	1:2000	1:500

NOTE: (1) The wide discrepancy between the last two columns seemed to be due to differences in absorbability; (2) concentrations of 1:30000, perfused, lengthened the refractory period in a few experiments; (3) Santesson, frog's heart, found 1:5000 to be very effective, 1:20000 appreciably active, and 1:30000 inactive.

EXPERIMENTAL DATA

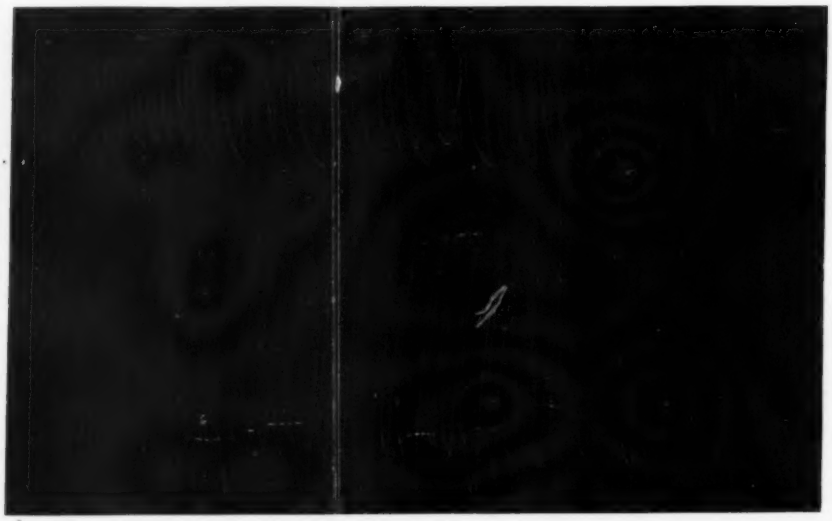
Direct observation was always combined with the use of recording apparatus. The most conspicuous phenomena exhibited by the reptilian heart after the administration of quinidine were alterations of rhythm and amplitude. The explanation of these effects was sought for in the nervous mechanism, the myocardium, and the conducting system.

Changes in Rhythm.—The effect of quinidine on the rhythm of the heart was determined for the whole heart and for the auricle and the ventricle, separately. The changes may be grouped under the captions of (a) retardation, (b) irregularities of rhythm, and (c) cardiac arrest.

(a) Retardation. The rate of both chambers was reduced in proportion to the tension of the drug. Quantitatively the effect was somewhat greater on the ventricle than on the auricle, on which in turn it was greater than on the sinus. The lowest effective concentrations (1:10,000, perfused) produced simultaneous slowing of both chambers; intermediate ones, greater slowing, slowing with group formation, or a ventricular rate of ½ to ⅙ of the auricular; massive doses caused stoppage of the heart, as will be noted below. With the exception of the last mentioned, these effects were always removable, in the case of the perfused heart, by withdrawing the drug.

(b) Irregularities. The irregularities observed were missed beats, group formation, premature systole, flutter and fibrillation. The first two were of common occurrence and often appeared simultaneously in auricle and ventricle in experiments on the whole heart. They were also exhibited, however, by excised strips of both chambers. In our experience, premature systole was not of very frequent occurrence. It was observed most often in the case of the ventricle. Flutter and fibrillation were produced occasionally as a preliminary to some other irregularity or in alternation therewith. None of these irregularities appeared after minimal doses. Most frequently they were seen to follow rapidly high tensions of the drug. At times, however, they were presented during the resumption of rhythm in a heart resuscitated after a massive dose. Disappearance was the rule on withdrawal of the drug.

(c) Cardiac Arrest. The administration of massive doses (1:2000, perfused) produced within a few seconds usually diastolic, but occasionally systolic, arrest. In both of the species examined, the ventricle stopped under the influence of lower concentrations than did the auricle. There was at times a preliminary slowing with dropping of beats or group formation, but



Tracing 1.—Heart *in situ*. Turtle. Slowing with group formation after two and one-half mg. of quinidine per liver.

Tracing 2.—Heart, excised. Turtle. Premature systole after perfusion with quinidine (1:2000).

usually there was no prodrome. There was rarely exhibited a decrease in the amplitude of the ventricular excursions; cessation was usually ushered in by a sharp increase in the diastolic relaxation. The auricle on the other hand came more gradually to its standstill, the amplitude slowly decreasing to a mere fraction of the original. The sinus continued to pulsate long after

total quiescence of the chambers. A change in its rhythm was not observed even with doses which stopped both auricle and ventricle. While less irritable, the arrested heart still responded to mechanical, electrical, and sympathetic stimulation (epinephrine). Removal of the drug (perfusion experiments) was followed by a resumption of the rhythm, the amplitude and rate gradually approximating the normal. Dropped beats and group formation were common during the recovery. In some cases, we stopped the heart and revived it as often as five times. The cardiac tissue did not seem to be permanently injured as after chloroform, but rather to be physiologically depressed.

Most of the above alterations of rhythm have been recorded by other observers, experimental and clinical. Among the former, it may be noted that Santesson[2] working on the frog's ventricle observed usually a slower rate, which irregularly increased and decreased; de Arric,[6] slowing by all doses in the case of the turtle; Arrilliga *et al*[7] on the dog, a transitory

Tracing 3.—Heart, excised. Turtle. Acute arrest of the ventricle after quinidine (1:2000), and recovery after removal of the drug.

tachycardia passing over quickly into bradycardia; Korns,[4] a retardation in the dog and guinea pig with temporary increase after single doses. Clinically, Levy[8] refers to a ventricular tachycardia on pressing the administration of the drug; Wolferth,[9] to ventricular slowing subsequent to a preexisting tachycardia; and Lewis,[10] to slowing of the auricle accompanied by quickening of the ventricle. Our data obtained from presumably normal hearts showed slowing without the preliminary quickening described for the pathological; quiescence was never preceded by a tachycardia.

Changes in Amplitude.—The observations in respect to changes in amplitude were made in the course of other determinations. Some of these are shown in Tracings 1, 3, 4, 5, and 9. Massive doses decreased the amplitude of both chambers, the alteration being usually systolic. The effect on the ventricle was more immediate and profound, and that on the right side was greater than on the left. Intermediate doses as a rule increased the amplitude of both

auricle and ventricle; systole and diastole both shared in the change. An increase in amplitude was usually the accompaniment of some alteration in rhythm. In fact, it commonly ushered in slowing or group formation, an exaggeration of diastole being repeatedly the prodrome. Our observations are in accord with those of Santesson[2] on the frog, of de Arric[6] with the cinchona alkaloids on the turtle, and of Cohen and Levy[11] on the dog. They are at variance, however, with those of Boden and Neukirch[12] on the rabbit.

Tone changes as a separate entity were never conspicuous. They were usually observed only after massive doses and were then for the most part in the direction of the diastole.

Changes in the Nervous Apparatus.—The portions of the nervous apparatus to which attention was given were the endings of the vagus and the sympathetic. Our observations were made on the *in situ* and perfused hearts.

(I) *The Vagus.* We examined the effect of quinidine on the tone of the vagus and that of atropine on the quinidine reaction.

(a) The Tone of the Vagus. The minimal stimulus to slow and just stop the heart was determined before the administration of quinidine and during the height of its action, and just after recovery from alterations of rhythm. It neither raised nor lowered the threshold to electrical stimulation.

(b) Effect of Atropine. Atropine was administered in dosage sufficient to paralyze the vagus endings, both before and during the action of quinidine. In this dosage, it did not alter the action of quinidine qualitatively or quantitatively. The heart was slowed as previously and other alterations in rhythm were as conspicuous as before.

Santesson[2] was unable to detect any effect on the vagus in frogs. But Lewis *et al*[3] and Korns[4] observed in dogs and guinea pigs a striking paretic effect at the periphery. It should be noted that the latter were working with intact animals, while Santesson and ourselves were using ones in which the central connections had been destroyed.

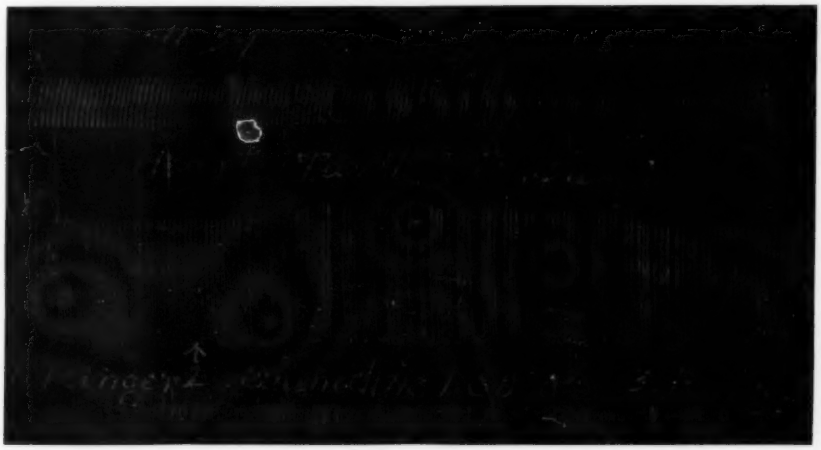
(II) *The Sympathetic.* We examined the effect on the sympathetic through the use of epinephrine. Quinidine was administered in dosage, severally, to produce slowing, blocking, and stoppage. Epinephrine was then administered during the continuance and after the withdrawal of quinidine. In the first case, it quickened the rate, restored the A-V sequence, and retarded stoppage, larger doses of quinidine being necessary to effect these changes. Further, group formation disappeared, often with an exaggeration initially of the phenomenon of extraventricular systole. Administration after the withdrawal of quinidine, hastened the recovery of the normal rhythm. On the basis of the known action of epinephrine, we may state that sympathetic stimulation is antagonistic to quinidine action and that quinidine acts independently of the sympathetic endings.

Changes in the Myocardium.—Attention was given to the following properties of the myocardium: (a) Irritability, (b) Elasticity, (c) Latent Period, (d) Refractory Phase and (e) Conduction. The determinations in respect to the above were made for both auricle and ventricle on *in situ* and perfused hearts.

(a) Irritability. The minimal stimulus, with break induced shocks, was determined for the unpoisoned tissue and then this strength of current was employed as the standard of comparison after quinidine in various dosages. The stimulus was delivered with a bipolar platinum electrode applied to the surface of the chamber under examination. Care was taken not to stimulate too frequently and to change the position of the electrode from time to time.

After all dosages the musculature responded less readily to electrical stimulation. Not only was a stronger current necessary to produce a contraction, but the response was more sluggish. But, even after standstill doses, high intensity of current could effect contraction, provided the tissue had not remained too long in contact with the drug. Incidentally, strong mechanical stimuli were also effective. Except after prolonged contact with massive doses, liberal lavage for a few minutes was followed by restoration of irritability or increase to approximately normal.

Arrilliga[7] has reported decreased irritability in the dog and frog, while



Tracing 4.—Heart, excised. Turtle. Increased amplitude after perfusion with quinidine (1:5000).

Boden and Neukirch[12] have observed in rabbits a decreased tendency toward artificially produced flutter, and other experimenters[11] greater difficulty in producing fibrillation. We have found the decrease in irritability to be shown somewhat more markedly in the ventricle than in the auricle, and it seems reasonable to attribute to it, in part at least, the phenomenon of missed ventricular beats.

(b) Elasticity. We refer here to the speed or rapidity with which the tissue on being distended or elongated resumed its original cross-section or length. Evidence of influence of quinidine on elasticity was determined by observing the "spring" with which the muscle recovered from the descent ("fling") below the base-line. In the unpoisoned this was immediate, a very acute angle being described; after quinidine the angle was much wider, the arc of recovery being continued into the systolic rise. Further the "fling" was much greater. This may be interpreted as indicating greater loss of tone during diastole, due to which the light lever stretched the tissue more

than normally, and decrease in elasticity, due to which it recovered from the fling less quickly. Decreased elasticity has been reported by Santesson[2] for the frog's heart.

(c) The Latent Period. We measured the interval between the application of a stimulus and the resulting contraction. The stimulus was applied with platinum electrodes attached to the base of the aorta and the tip of the chamber under examination. Minimal, break, induced shocks were employed. The time interval was recorded with a tuning fork of 100 vibrations per second. The following apparatus was used to mark the limits of the period: Two levers, A and B, of equal length were arranged to move about a common fulcrum, one recording at a higher level than the other. A was attached to the apex of the chamber being studied; B was laid on the armature of an electromagnet, connected in series with the primary of the induction coil. The apparatus is shown in diagrammatic form in Fig. 2.

The latent period was lengthened by all concentrations and dosages of quinidine, almost instantaneously by the highest and after the lapse of about three minutes following the lowest. In the perfused heart, following the lower concentrations, perfusion with Ringer's fluid for a few minutes

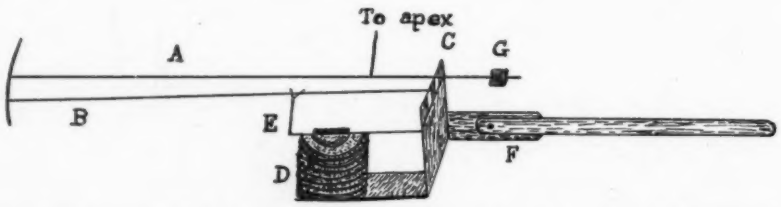


Fig. 2.—Diagram of signal. *A*, cardiac lever; *B*, signal lever; *C*, fulcrum; *D*, electromagnet; *E*, armature; *F*, fine adjustment; *G*, counterpoise. Since *A* and *B* are of equal length and move around the same axis, the opening and closing of the primary is always recorded on the same arc as the cardiac contractions.

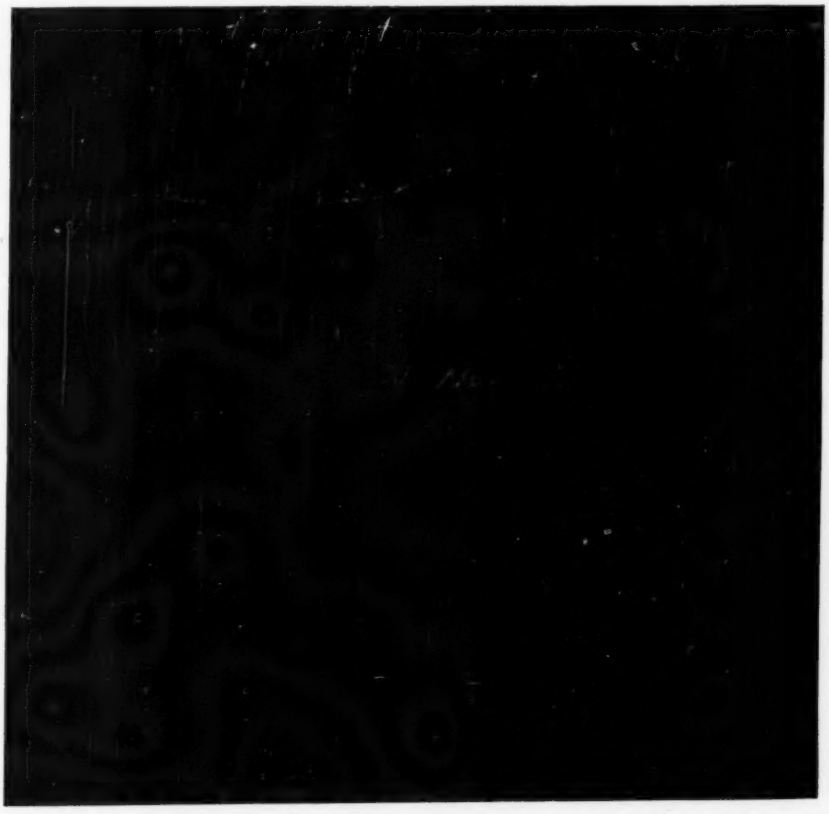
reduced the period to approximately its original length, while repetition of quinidine again lengthened it. With the smaller doses, then, the effect was a temporary one, dependent on the continued presence of the drug.

(d) The Refractory Phase. In the *in situ* heart, the stimulus was applied with a bipolar platinum electrode; in the perfused, one pole was attached to the base of the aorta and the other to the tip of the chamber, as in the examination of the latent period. The immersed strips of cardiac muscle were stimulated from end to end. The apparatus for determining the exact point in the cardiac cycle at which the stimulus was delivered has been described in a previous paper by Waddell.[13] It consisted of an electric signal so arranged as to pull the writing point away from the recording surface at the instant of making or breaking the primary circuit.

A marked increase in the length of the refractory phase of both auricle and ventricle was exhibited. This increase was roughly proportional to the dosage or concentration of the drug. It was usually in evidence within a few seconds after the administration. The end of the refractory period was extended, in proportion to the "normal" 3 to 10 times as far into diastole. Removal of the drug was rapidly followed by a return to about

the original length, even in case of very profound alteration with a final picture of cardiac arrest. The phenomenon may be repeatedly produced and abolished.

In our hands, the lengthening of the refractory phase was the most delicate reaction of quinidine. It was demonstrated after a concentration as low as 1:30,000, which was inactive in every other respect. Among other investigators, the increase has been observed by Santesson[2] on the frog, Arrilliga *et al*[7] on the dog, and Korns[4] on the guinea pig. None of them have, however, emphasized the sensitiveness of the reaction.



Tracing 5.—Ventricle, excised. Turtle. Lengthening of the refractory period after perfusion with quinidine (1:10000). The normal gives a second contraction near the apex, while the poisoned does not respond until three-fourths of diastole has been completed.

(e) Conduction. The influence of quinidine on conduction was studied on the *in situ* and the perfused heart. Attention was given to changes in the auriculoventricular interval and to the phenomenon of block.

A-V Interval. In making detreminations relative to the length of the A-V interval, simultaneous tracings were made from the auricle and the ventricle with levers of equal length, moving about the same horizontal axis. The interval was measured with a tuning fork of 100 vibrations per second. The time was lengthened in the perfused heart with concentrations as low as 1:20,000, and in the *in situ* with doses as small as one mg. Whereas

in the unpoisoned heart, auricular systole usually began after the previous ventricular systole had been completed, so that a distinct pause was in evidence, after quinidine due to tardiness on the part of the ventricle, auricular systole began during the ventricular diastole of the last cycle.

Block. After intermediate and large dosage (3-6 mg. per liver; 1:3000-1:1000, perfused) auriculoventricular (and rarely intraventricular and sino-auricular) block was exhibited. The last two were observed only after the largest dosage. The first was usually preceded by irregularity, group formation, or great slowing of the whole heart; at times it was initiated by no other phenomenon than an excessively deep ventricular diastole. The block was at first of low grade (2:1 or 3:1) but progressively increased under

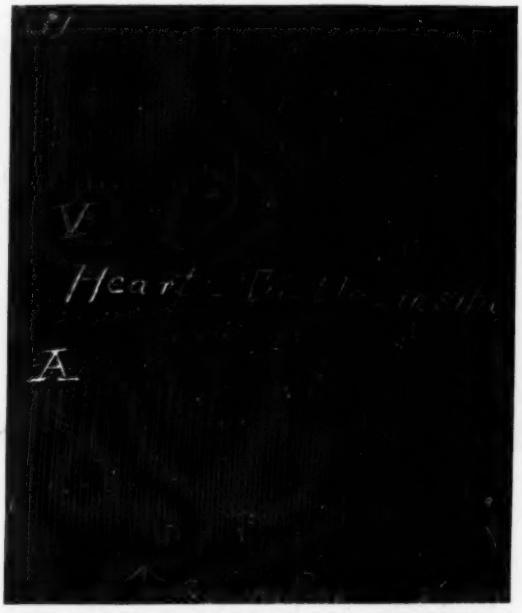
Tracing 6.—Heart *in situ*. Turtle. Lengthening of the auriculoventricular interval after quinidine (1:1000). The average duration in the normal was 0.34 sec.; in the poisoned, 0.52 sec.

the influence of high dosage until complete block was established or the ventricle ceased to pulsate. In the case of the excised heart, prolonged lavage with Ringer's fluid generally removed the block, while repetition of the administration of quinidine restored it.

Santesson[2] speaks of the sinus and auricle being faster than the ventricle, so that the impulse to ventricular contraction misses. He does not refer to block, though his tracings show every indication that the phenomenon was present. During recent years, decreased auriculoventricular conduction has been noted by numerous investigators, among them Cohn and Levy,[11] Lewis,[10] Schott,[14] Wolferth,[9] and Korns.[4] Wolferth points out that the

conducting system is very susceptible to quinidine, the action being on the muscle bundles. Korns emphasizes a retardation of the intraauricular and intraventricular conduction, stating that depression of A-V conduction is never conspicuous as an individual effect. In the reptilian heart, as we have shown, it is a very acutely produced phenomenon, which may appear suddenly without prodrome.

Korns[4] and also Lewis *et al*[3] have particularly called attention, on the other hand, to the appearance of intraauricular and intraventricular block—retardation of the impulse in a branch of the conducting bundle. While on the whole our procedures were not adapted to the study of branch conduction, we noted instances of alteration in the case of the ventricle. For, now and then, after high concentrations in the perfused heart, there developed a lag in the beginning of systole in one of the ventricles as compared with the



Tracing 7.—Heart *in situ*. Turtle. Auriculoventricular block after two and one-half mg. of quinidine per liver.

other. Most often this was shown in the left, simultaneous tracings recording the systole of this ventricle as beginning even as late as the end of systole in the right. Conceivably this was due to a retardation of the impulse through the branch to that chamber.

All the reactions of quinidine on the reptilian heart point to its being a direct but nonspecific depressant. The changes in rate, irritability, elasticity, refractory phase, and conduction are all in favor of this view; as is also the observation that it likewise depresses the activity of other rhythmical tissue (vagina, esophagus, and intestine[15]). The increase in amplitude occurring in certain instances and the quantitatively greater effect shown on the ventricle are against it only apparently; for the former may be explained on the basis that the greater interval between the contractions gives more time

for recuperation and the latter on the basis of differences in the blood supply to the auricle and ventricle.

This depression is not due to destructive action on the tissue. It has been noted that the effects of quinidine depend on the continued presence of the drug. They are abolished by its withdrawal. Permanent cardiac arrest after prolonged contact with high concentrations is not opposed, since the suppression of activity would lead to the development of a vicious circle through the introduction of asphyxia.

That it most probably acts through depressing metabolic activity is not theorizing too far. In favor of this are the following: (1) Quinine, its isomer, is known to possess this action in respect to nitrogenous metabolism, and (2) changes similar to those produced by quinidine may occur in hearts impoverished by rapid exsanguination.[16]

Tracing 8.—Heart, perfused. Turtle. Slowing of the ventricles, followed by flutter with the right ventricle about twice as rapid as the left. A lagging of the left ventricle is seen at the end.

SUMMARY

1. The effect of quinidine has been shown on the reptilian heart as follows: (a) *in situ* with superficial, intravenous, intrahepatic, and subcutaneous administration; (b) excised whole and separated chambers, perfused; (c) strips of the auricular and ventricular muscle, immersed.

2. The effects are qualitatively the same on auricle and ventricle, but quantitatively greater on the latter.

3. The rate is slowed; massive doses produce arrest, usually diastolic.

4. The amplitude of the excursions is increased; with massive doses there is a decrease, which may be systolic or diastolic.

5. The diastolic tone is decreased, but the change bears no definite relationship to dosage.

6. The irritability of the myocardium is decreased but even standstill doses do not totally abolish responsiveness to electrical and mechanical stimulation.

7. The elasticity of the heart muscle is decreased, as is shown by tardy recovery from stretching.

8. The latent period on electrical stimulation is lengthened.

9. The refractory phase is lengthened. This is the most delicate reaction of the drug.

10. Conduction is constantly disturbed, leading to increase in the A-V interval and to atrioventricular block; rarely to sinoauricular.

11. Parasympathetic depressants (atropine) do not alter the reaction in any of its details.

12. Sympathetic stimulants (epinephrine) are still effective after quinidine.

13. Other rhythmical tissue (vagina, intestine, esophagus) is likewise depressed by quinidine.

14. The effects of quinidine are quickly removed by withdrawal of the drug.

CONCLUSIONS

1. Quinidine produces its effects through action on the musculature of the heart, including that of the conducting system.

2. With moderate dosage, there is no evidence of permanent tissue damage, all effects being abolished by withdrawal.

3. Quinidine seems to be a physiological depressant, analagous to ether on the brain and cocaine on nerves.

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THE WASSERMANN TEST IN RABBIT SYPHILIS*

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THE Wassermann reaction has received very little attention by investigators in the field of experimental syphilis, as a review of the literature readily shows. It has been observed that normal rabbits sometimes react negatively and at other times the same animal reacts positively to the test, and for this reason there has been great skepticism regarding the value of this reaction in rabbit syphilis. Blumenthal[1] in 1911 conducted a series of experiments on the Wassermann reaction in experimental rabbit syphilis. He used the old Wassermann technic and by reducing the amount of antigen used to one-half and the serum to one-quarter, he obtained negative reactions in 42 normal rabbits. On applying this modification of the test to 56 rabbits inoculated in the testis and the anterior chamber of the eye, the Wassermann reaction was negative regardless of the clinical symptoms. Of ten animals which developed generalized syphilis following inoculation by the intravenous or intracardiac method of Uhlenhuth-Mulzer, five showed a strongly positive reaction. Two were negative and three slightly positive.

In our work[2] the antisheep system and active serum were used throughout. Fresh, pooled guinea pig serum was used as complement and serum from immunized rabbits was used as amboceptor. Noguchi's acetone-insoluble extract of heart muscle was employed as antigen. The amount of antigen used was 0.1 c.c. of a 5 per cent emulsion, carrying 8 antigenic units; it was not anticomplementary nor hemolytic in ten times the amount used in the test.

The titration is run according to the following plan. Test tubes are set up in four rows with 5 tubes in each row, as follows:

Row "A," to determine the amount of native complement present in each dilution of serum;
Row "B," serum plus one unit of complement;
Row "C," antigen plus one unit of complement;
Row "D," antigen plus two units of complement.

After the blood to be tested is withdrawn, clotting is permitted to take place at room temperature; it is then placed in the ice box overnight. After centrifugalization the serum is diluted as follows: 2.5, 5, 10, 20, and 50 per cents of each solution are used in the test. Table I will illustrate the method used.

*This investigation was a part of the work done at the University of Wisconsin and the Wisconsin Psychiatric Institute in the development of new remedies for the treatment of nervous syphilis; it was planned and supervised by Dr. W. F. Lorenz and Dr. A. S. Loevenhart. The expense of this research was partially defrayed by a grant from the Public Health Institute of Chicago, Illinois.

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TABLE I

D	2.5% serum 0.1 c.c. Antigen 2 units complement	5% serum 0.1 c.c. Antigen 2 units complement	10% serum 0.1 c.c. Antigen 2 units complement	20% serum 0.1 c.c. Antigen 2 units complement	50% serum 0.1 c.c. Antigen 2 units complement
C	2.5% serum 0.1 c.c. Antigen 1 unit complement	5% serum 0.1 c.c. Antigen 1 unit complement	10% serum 0.1 c.c. Antigen 1 unit complement	20% serum 0.1 c.c. Antigen 1 unit complement	50% serum 0.1 c.c. Antigen 1 unit complement
B	2.5% serum 0.1 c.c. 1 unit complement	5% serum 0.1 c.c. 1 unit complement	10% serum 0.1 c.c. 1 unit complement	20% serum 0.1 c.c. 1 unit complement	50% serum 0.1 c.c. 1 unit complement
A	2.5% serum 0.1 c.c.	5% serum 0.1 c.c.	10% serum 0.1 c.c.	20% serum 0.1 c.c.	50% serum 0.1 c.c.

The above set of tubes with their contents is incubated at 37.5° C. for 30 minutes, after which 1 c.c. of 1½ per cent sheep-corpuscle suspension is added to each tube, and then incubated again for 30 minutes. After the second incubation the amboceptor is added, and the whole incubated for one hour with frequent agitation of the tubes. The reaction is now ready to read according to the degree of hemolysis. This method is used by Lorenz in determining the therapeutic effect of treatment upon the serology in man.

During the past several years we have submitted many nonsyphilitic rabbits under observation in the laboratory to this test, and in none of them has a positive reaction been observed. In one series of normal rabbits, tests were made at weekly intervals for a period of three weeks without a positive reaction in any case.

Having determined to our satisfaction that normal animals consistently react negatively to this test, we began to test syphilitic animals according to the same procedure. The animals were inoculated into the testicle according to the method of Brown and Pearse.[3] From a series of 39 syphilitic rabbits tests were made at varying intervals, positive reactions occurring in every instance. A few of these animals developed generalized lesions, but in the great majority the clinical course remained local. In one animal inoculated intravenously generalized lesions occurred, and a strongly positive reaction developed.

The cyclic reaction which characterizes the course of the syphilitic infection in rabbits, and the sudden changes which sometimes occur in the course of the local reaction, suggested the idea of following the clinical course of the infection in connection with the Wassermann test. Seven animals were selected in which the local cycle went to completion without therapeutic intervention. The first test was made when clinical symptoms developed, and the last test when the testicle had apparently returned to normal.

The following case histories show the course of the clinical manifestations and the Wassermann reaction:

RABBIT 334. FEMALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Nov. 2, 1922	Intravenous inoculation	
Dec. 15, 1922	Wassermann −	
Jan. 10, 1923		Gummas on anus, right upper eyelid and skin of left popliteal space
Jan. 12, 1923	Wassermann +	
Jan. 16, 1923	Wassermann ++	Additional lesions on rt. front foot and at base of left ear
Jan. 26, 1923	Wassermann ++++	
Feb. 2, 1923	Wassermann ++++	
Feb. 5, 1923		All gummata healed except that on anus. Opacity of cornea developed
Feb. 9, 1923	Wassermann +++	
Feb. 18, 1923	Wassermann +	
Feb. 19, 1923		All lesions healed
Feb. 23, 1923	Wassermann −	

RABBIT 350. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Dec. 6, 1922	Intrascrotal inoculation	
Jan. 17, 1923		Point of inoculation slightly hyperemic and indurated
Jan. 22, 1923		Lesions appeared on eyelids
Jan. 27, 1923	Area of scrotal induration excised	Dark-field showed many actively motile organisms
Feb. 12, 1923		Chancre formed at scar
Feb. 19, 1923		Lesions developed on the anus, on the tail opposite the scrotum, and on both front legs
Feb. 22, 1923	Wassermann −	Lesions on both scrota, anus, tail, front legs and both eyes
Mar. 1, 1923	Wassermann +++	Lesions progressing
Mar. 8, 1923	Wassermann +++	
Mar. 15, 1923	Wassermann ++++	
Mar. 19, 1923		Induration subsiding and crusts loosening
Mar. 29, 1923	Wassermann ++	
Apr. 4, 1923	Wassermann ++	
Apr. 9, 1923		Animal died. Chancre crust on right testis and papule on right upper eyelid. Other lesions healed

RABBIT 364. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Jan. 8, 1923	Intratesticular inoculation	
Jan. 29, 1923		Gumma 5 mm. in diam. in right testis
Feb. 2, 1923	Wassermann −	
Feb. 12, 1923		Entire testis indurated
Feb. 15, 1923	Wassermann ++++	
Feb. 22, 1923	Wassermann +++	
Feb. 26, 1923		Chancre 1.5×3 cm.
Mar. 1, 1923	Wassermann ++++	
Mar. 8, 1923	Wassermann ++	
Mar. 15, 1923	Wassermann −	
Mar. 26, 1923		Lesion is retrogressing
Mar. 29, 1923	Wassermann −	
Apr. 12, 1923	Wassermann −	
Apr. 30, 1923		Complete healing

RABBIT 369. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Jan. 8, 1923	Right intratesticular inoculation	
Jan. 29, 1923		Right testis slightly swollen but no induration
Feb. 1, 1923	Wassermann −	
Feb. 5, 1923		Right testis entirely indurated. No chancre
Feb. 15, 1923	Wassermann −	
Feb. 22, 1923	Wassermann +	No chancre. Intense induration
Mar. 1, 1923	Wassermann +++	No change in lesion
Mar. 8, 1923	Wassermann +++	
Mar. 15, 1923	Wassermann +++	No change in lesion
Mar. 29, 1923	Wassermann −	
Apr. 9, 1923		Lesion healed

In this instance the Wassermann reaction remained negative after marked induration developed, but became positive although no chancre developed.

RABBIT 372. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Jan. 8, 1923	Right testicular inoculation	
Jan. 29, 1923		Lesion began with edema of scrotum
Feb. 22, 1923	Wassermann +++	Intense induration. No chancre
Feb. 26, 1923		Intense induration and development of chancre
Mar. 1, 1923	Wassermann ++++	
Mar. 5, 1923		Entire scrotum necrotic with intense induration
Mar. 8, 1923	Wassermann ++++	
Mar. 15, 1923	Wassermann +++	
Mar. 29, 1923	Wassermann +	
Apr. 2, 1923		Chancre 2×2.5 cm. Intense induration. Lesion not progressing
Apr. 12, 1923	Wassermann +	
Apr. 26, 1923	Wassermann −	
Apr. 30, 1923		Lesion entirely healed
May 24. 1923	Wassermann −	

RABBIT 375. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Jan. 15, 1923	Right testicular inoculation	
Jan. 29, 1923		Right testicle firmly indurated
Feb. 22, 1923	Wassermann ++	Right testis and inguinal glands indurated
Mar. 1, 1923	Wassermann +++	
Mar. 7, 1923	Wassermann +++	
Mar. 14, 1923	Wassermann +	
Mar. 19, 1923		Induration more intense and chancre appeared
Mar. 26, 1923		Chancres enlarging. Intense induration
Apr. 16, 1923		Lesions not progressing
Apr. 26, 1923	Wassermann ++	
May 3, 1923	Wassermann +	
May 11, 1923	Wassermann +	
May 24. 1923	Wassermann −	Testis still indurated; healing has begun
Sept. 17, 1923		Slight induration persists

RABBIT 393. MALE, NO TREATMENT

DATE	PROCEDURE	REMARKS
Feb. 17, 1923	Right testicular inoculation	
Feb. 28, 1923		Right testis slightly indurated
Mar. 1, 1923	Wassermann −	Right testis slightly indurated
Mar. 7, 1923	Wassermann +	
Mar. 14, 1923	Wassermann +	
Mar. 19, 1923		Right testis becoming edematous
Mar. 25, 1923	Wassermann +	
Mar. 26, 1923		Intense induration. Chancre 2×3 cm.
Apr. 5, 1923	Wassermann +	
Apr. 16, 1923		Lesion not progressing. Induration intense
Apr. 18, 1923	Wassermann ++	
May 8, 1923	Wassermann +++	
May 29, 1923	Wassermann −	
June 18, 1923		Chancre healed. Testis entirely soft

It is evident, therefore, that in our hands the Wassermann reaction, performed as outlined, becomes positive with the appearance of clinical manifestations of the disease and recedes as the lesions heal. In most cases the development of the Wassermann reaction parallels in a general way the lesions, while in other instances the reaction first becomes positive a month or more after the lesions appear. These results indicated that the test might be used as an aid in the determination of the therapeutic value of new arsenicals. Accordingly, we observed the effect on the Wassermann reaction of 3,4-digluconyl-diamino-phenylarsonic acid, first prepared by Lewis and Hamilton[3] which showed some therapeutic value.* A few cases in which it was used are described here.

RABBIT 370. MALE, RABBIT 372 SERVED AS CONTROL

DATE	PROCEDURE	REMARKS
Jan. 8, 1923	Right testicular inoculation	
Jan. 29, 1923		Initial lesion consisted of extreme edema of scrotum
Feb. 1, 1923	Wassermann ++++	
Feb. 5, 1923		Edema of scrotum. Scrotal ulcer, 5 mm. in diam.
Feb. 8, 1923	Wassermann ++++	
Feb. 14, 1923	Treated with 500 mg. per kilo of drug	Scrotal ulcer 1 cm. in diam. Inguinal glands indurated
Feb. 15, 1923	Wassermann +++	
Feb. 22, 1923	Wassermann +++	
Mar. 1, 1923	Wassermann ++	
Mar. 8, 1923	Wassermann −	
Mar. 9, 1923	Treated with 500 mg. per kilo of drug	Testis softer. Ulcer 2×2.5 cm. Inguinal glands indurated
Mar. 15, 1923	Wassermann −	
Mar. 29, 1923	Wassermann −	
Apr. 4, 1923		Chancre healed. Testis fibrous
Apr. 12, 1923	Wassermann −	

*We desire to thank Drs. W. L. Lewis and C. S. Hamilton for furnishing us with the compound used.

RABBIT 353. MALE, RABBIT 350, REPORTED ABOVE, SERVED AS CONTROL

DATE	PROCEDURE	REMARKS
Dec. 6, 1922	Intracutaneous scrotal inoculation	
Jan. 10, 1923		Lesion commenced with hyperemia and induration at point of inoculation
Feb. 8, 1923	Treated with 400 mg. per kilo. of drug	Palpable lymph gland. Chancre 1.9 cm. diam.
Feb. 22, 1923	Wassermann ++	
Feb. 26, 1923		Crust 7 mm. in diam. Lymph gland soft
Mar. 1, 1923	Wassermann ++	
Mar. 8, 1923	Wassermann +	
Mar. 9, 1923	Treated with 400 mg. per kilo of drug	Testis softer
Mar. 15, 1923	Wassermann ++	
Mar. 19, 1923		Lesion healed
Mar. 29, 1923	Wassermann −	
Apr. 26, 1923	Wassermann −	

RABBIT 377. MALE. RABBIT 375 SERVED AS CONTROL

DATE	PROCEDURE	REMARKS
Jan. 15, 1923	Right testicular inoculation	
Feb. 12, 1923		Scrotum became extremely edematous with slight induration of testis
Feb. 25, 1923	Wassermann ++++	
Mar. 21, 1923	Treated with 750 mg. per kilo. of drug	Large chancre had developed, 10-12 mm. Testis indurated
Mar. 29, 1923	Treated with 750 mg. per kilo. of drug	Testis softer
Apr. 5, 1923	Wassermann +	
Apr. 19, 1923	Wassermann −	
Apr. 20, 1923		Chancre healed. Testis soft
May 11, 1923	Wassermann −	

RABBIT 374. MALE. RABBIT 375 SERVED AS CONTROL

DATE	PROCEDURE	REMARKS
Jan. 15, 1923	Right intratesticular inocula-	
Feb. 12, 1923	tion.	Lesion commenced with edema of scrotum
Feb. 22, 1923	Wassermann +++	Edema of scrotum. Induration of testis
Feb. 29, 1923	Wassermann ++++	
Mar. 5, 1923		Entire lower surface of scrotum was ulcerated, testis indurated
Mar. 6, 1923	Wassermann ++++	Large scrotal ulcer. Testis indurated
	Treated with 1.0 gm. per kilo. of drug	
Mar. 13, 1923	Wassermann +++	
Mar. 16, 1923	Treated with 1.0 gm. per kilo. of drug	Slight softening of testis
Mar. 28, 1923	Wassermann −	
Apr. 9, 1923		Testis slightly fibrous
Apr. 26, 1923	Wassermann −	
May 24, 1923	Wassermann −	

CONCLUSIONS

From these observations we conclude that nonsyphilitic normal rabbits always react negatively to the Wassermann reaction when performed as above described. Rabbits successfully inoculated with syphilis always de-

velop positive Wassermann reactions. In general, the Wassermann reaction becomes positive when clinical signs of syphilis appear, but it may be delayed a month or more. The Wassermann reaction parallels in a general way the course of the local reaction, becoming negative in untreated animals during the period of regression. In treated animals the Wassermann reaction usually becomes negative before the complete disappearance of clinical manifestations.

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THE EFFECT OF THE GAS FLAME IN THE ADJUSTMENT OF THE REACTION OF STERILE SOLUTIONS*

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IN ATTEMPTING to adjust certain sterile solutions including Locke's solution, 0.9 per cent NaCl and others, to given H-ion concentrations between pH 7.0 and pH 8.0, much difficulty was encountered in obtaining a constant reaction. After adding the calculated amount of acid or alkali, the reaction was usually found to be less alkaline than expected, and a further change toward acidity frequently occurred. This change in reaction was found to be due to the absorption of CO_2 from the gas flame with the consequent formation of H_2CO_3 in the solution. While it may seem that such a simple and well-recognized chemical phenomenon should hardly merit mention, the authors have failed to find any reference to this subject in the literature on adjustment of reaction in solutions. It was therefore felt that the following observations were of sufficient importance to deserve recording.

Locke's solution adjusted to pH 7.3 with a mixture of N/10 NaOH and N/10 $NaHCO_3$, was distributed in 50 c.c. amounts into 100 c.c. Erlenmeyer flasks. Gelatin-Locke's solution† was similarly adjusted to pH 7.5 and likewise distributed. The flasks were then briefly flamed over a gas burner 5 to 6 times for 1-2 seconds at each flaming. Immediately afterwards, the pH was retested and the results obtained were as shown in Table I.

Table I

SOLUTIONS USED	pH OF SOLUTION		DIFFERENCE IN pH
	BEFORE FLAMING	AFTER FLAMING	
Locke's	7.3	6.8	0 5
Gel. Locke's	7.5	7.0	0.5
Gel. Locke's	7.5	7.1	0.4

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†One-eighth per cent gelatin in Locke's solution.

TABLE II

KIND OF SOLUTION	ADJUSTED WITH		BUFFERED WITH PHOSPHATE	pH OF SOLUTION						DIFFERENCE IN pH BEFORE AND IMMEDIATELY AFTER FLAMING
				BEFORE FLAMING	IMMEDIATELY AFTER FLAMING	AT END OF 1 HR.	AT END OF 2 HRS.	AT END OF 4 HRS.	AT END OF 24 HRS.	
Locke's Solution	A	NaOH	0	7.7	6.7	7.0	7.1	7.4	7.5	1.0
	B	NaOH & $NaHCO_3$	0	7.7	7.1	7.2	7.3	7.4	7.5	0.6
	C	NaOH & $NaHCO_3$	1:50	7.6	7.2	7.3	7.3	7.4	7.5	0.4
Gelatin-Locke's Solution	A	NaOH	0	7.9	7.1	7.3	7.4	7.5	7.9	0.8
	B	NaOH & $NaHCO_3$	0	7.8	7.2	7.3	7.5	7.6	7.8	0.6
	C	NaOH & $NaHCO_3$	1:50	7.9	7.5	7.5	7.6	7.6	7.9	0.4

In order to be sure that the change in reaction was caused by the absorption of CO_2 and was not due to the presence of acid products in the gas, the above experiment was repeated, using an alcohol flame. In this case, the differences in pH before and after flaming were even greater—0.9 of a pH unit in gelatin-Locke's solution and 1.1 in Locke's solution. The possibility that heating alone might produce an effect on the reaction was tested by employing electric heat* for sterilizing the mouth of the flask. Changes in pH resulting from opening the flasks under these conditions were very slight, not more than 0.1 of a pH unit in most instances.

A solution which had become less alkaline or even acid in reaction upon flaming showed a gradual return to almost its original reaction after several hours' standing—due to the loss of the absorbed CO_2. It was found, furthermore, that the degree of change could be diminished by adjusting with $NaHCO_3$ and buffering as well with phosphate. The following experiment illustrates the initial and subsequent changes in reaction brought about by flaming Locke's and gelatin-Locke's solutions adjusted and buffered in different ways.

Gelatin-Locke's and Locke's solutions were prepared from fresh double distilled water and distributed into three equal portions (A, B, and C) and their reaction was adjusted as follows: Solution A was adjusted with N/10 NaOH only, B with a mixture of N/10 NaOH and N/10 $NaHCO_3$ (1.4), and C, in addition to the alkaline mixture used in B, was buffered with a standard M/15 phosphate mixture of pH 7.6, in proportion of 1:50 by volume.† The solutions were then distributed in about 50 c.c. quantities into 100 c.c. Erlenmeyer flasks and plugged with cotton. Each flask was then flamed six times as before, over a gas burner, being held obliquely over the flame at an angle of 45°. Immediately after flaming, the flask was gently agitated by whirling motion for 8-10 seconds and the reaction of the solution tested. It is seen in Table II that the greatest change in reaction occurred in the solutions adjusted with NaOH and containing no buffer, while those solutions containing both bicarbonate and phosphate showed the least change. Within four hours after flaming, the pH of all the solutions had shown a return well toward the original value. At the end of twenty-four hours the gelatin-Locke's solutions had regained their original H-ion concentration while the Locke's solutions were still slightly less alkaline than previously. Tests with gelatin-salt solution gave approximately the same results. Keeping the solutions at a temperature of 37° C. hastened this process.

It was also found that the angle at which the container was held during flaming made a considerable difference in the outcome of the reaction of the solution. With the container held almost parallel to the flame, the change in reaction was much less than if it were held at right angles with the flame.

*The mouth of the flask was passed between two coils of high resistance wire raised to white heat by an electric current.

†This concentration of phosphate is approximately equal to the percentage of inorganic phosphate in the blood plasma.

LABORATORY METHODS

ELEMENTS OF CHOICE IN THE PHOTOMICROGRAPHY OF STAINED MICROSCOPIC SECTIONS*

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INTRODUCTORY

SUCCESSFUL photomicrographs depend largely upon the element of choice, granting of course, that one must first master the rudiments of dark-room technic. A good photomicrograph is possible only if its creator is wise in his choice of apparatus, subject, stain, ray-filter, magnification, exposure-time, photographic plate, and printing paper. The object of this article is to point out some of the factors influencing the pathologist in the choice of these; it does not pretend to be a *vade mecum* of protomicrography, but should indicate many pitfalls that await the beginner in this branch of laboratory work, as well as more experienced photographers, who take up photomicrography because they are already proficient in the art of gross photography.

CHOICE OF APPARATUS

This depends largely upon how much one wishes to spend, and for what purpose the photomicrographs are intended—for publication, or private records. If one have a sufficient budget at one's disposal, the best apparatus is none too good, and this is of the horizontal type, with the source of light, parallelizing lenses, diaphragms, microscope, and bellows on one, rigid, horizontal optical bench. The large German instruments are usually on two tables, the bellows being carried on a separate stand, which has the disadvantage of being capable of displacement, as the bellows may be jarred, or moved out of alignment by being knocked against. This is really a slight factor, as the defect is not difficult to remedy. The great advantage of the rigid, one-bench stand is pointed out by Petrunkevitch (1920) in his article "Standardized Microphotography": the possibility of standardizing and marking the positions of the various parts of the apparatus, so that they may be reset whenever disturbed.

The less expensive instrument is usually vertical, several optical supply houses sell small photomicrographic outfits in which the bellows is swung over the microscope by an upright support. In these the source of light is usually a tungsten filament lamp of some sort, the rays of which are focussed

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upon the substage condenser by means of its mirror. This is a fertile element of error, for the mirror is easily displaced and the rays sent astray. It is better, in such cases, to mount the microscope and bellows-holder on a table in which there is a hole, the lamp beneath the table so that its rays come through this aperture directly into the condenser. The Leitz-Edinger is another form of vertical outfit combining a camera lucida, a projector, and a photomicrographic outfit all in one. The source of light is an electric arc mounted on an upright above the special microscope, below which is a bellows. The latter is racked up when focussing the object on the table-screen beneath it, the plate holder inserted in its slide, and the bellows brought down until the plate is in the same plane as that occupied by the screen, which has been removed.

The small vertical apparatuses have the following disadvantages: the plate-holders are usually small (4 x 5″); the source of light is comparatively weak and rather difficult to align; the bellows extension is not as easily standardized, as a slight change in the length of the short bellows is comparatively greater than a much more marked one in the longer horizontal outfit. The advantages are: the small space required, the cheapness, and the portability. One can keep such an instrument in the corner of one's laboratory where it may be used at a moment's notice.

Whichever type of apparatus be chosen, the alignment of its parts must be perfect. The method of aligning them varies so with the various types, that it would be futile to attempt to cover the subject here. A very concise set of directions will be found in the Eastman Kodak Company's pamphlet "Photomicrography" (1919). Suffice it to say that the beam of light must run through the center of everything, including that of the photographic plate; it must emerge from the center of the parallelizing lens of the lamp and be projected in a straight, unbent line through the intersection of the diagonals of the ground-glass finder in the bellows. Everything intervening between these two points must be brought into line with them, including the lenses and diaphragms of the horizontal optical bench, and the microscope in either case. If this be done, the lighting will be even and free from disturbing shadows. In the vertical outfits the electric bulb must be centered on the bullseye lens and at such a distance from it that it will give the maximum light, the rays emerging in the axis of the lamp and going directly to the center of the substage condenser. The lamp must also be at the proper distance from this condenser, so that its beam fills it entirely, in order to get the maximum illumination, which is not very intense at its best. Once the bullseye lamp is adjusted, it should be set in position and never moved, for it is a tedious task to center and focus this type of illuminator.

CHOICE OF LIGHT SOURCE

There is little doubt that the electric arc gives the best light for this purpose, and it is important that it be mounted in the steadiest possible type of clock-work lamp, for the flickering and consequent resetting of the non-automatic type is very annoying. The right-angle arc, in which the large,

positive carbon lies horizontally, the small negative carbon vertically, is the form in general use. Those arcs in which the carbons are about six and four millimeters in respective diameters are the most satisfactory, for they produce a small, relatively fixed, circular incandescent crater which serves as a more reliable light-source than does a larger, more diffuse and vagrant area of incandescence. In order to get the best results it is necessary to determine the polarity of the house-circuit, so that the negative and positive poles of the lamp always correspond to those of the push-socket in the wall. The horizontal carbon is made thicker than the vertical because it would burn away faster than the latter were they of the same diameter. If the poles of the push-plug be reversed, one will find the negative carbon (which is now positive) burning away too rapidly and the horizontal one always working out over it, throwing a double image of the crater on the condenser. If one sees this, the poles of the push-plug should be reversed end-for-end, and marked to avoid a repetition of the condition. To get an evenly-lighted field the carbons must be so adjusted that the flame of the arc impinges upon the extremity of the horizontal carbon and stays there; it should not be permitted to wander back and forth over an eliptical crater; if it does so, it means that the positive carbon should be drawn back until a steady light is secured.

The usual light-source for the small vertical outfits is the coiled filament of a 6 to 8 volt Mazda bulb, mounted in a bullseye lamp. This filament must be carefully centered, so that its image falls at the center of the ground-glass finder of the camera. The disadvantage of this form of illuminator is the fact that the coils of the filament appear on the ground-glass as several diagonal, parallel lines of intenser light, surrounded by a halo of more diffuse radiance. It is possible to diffuse much of the bright lines by raising, or lowering the substage condenser, but when high-power photomicrographs are taken the bright lines become broad bands of light, the field being unevenly illuminated. A ground-glass bulb will help materially by further diffusing the image, but the light is proportionately diminished. The chief disadvantage of the tungsten filament light is its weakness, which necessitates long exposures for high-power pictures. During such long periods the apparatus is often shaken by the vibration of machinery in the building, or by passing cars or trucks, and the photographs blurred. Shock absorbers are provided on some of the small instruments, in the form of a rubber ball mount, which overcomes some of the difficulty, but even so the element of time is still to be dealt with. It takes a long time to complete a number of pictures if each exposure must be reckoned in minutes instead of seconds. Of course, they may be beautifully timed, because of the latitude allowed in such long exposures, but this is a doubtful advantage. Recently a light utilizing a small sphere of incandescent iridio-platinum (the "Point-o-light") has been devised, which does away with the parallel bands of the tungsten coil. I have had no personal experience with this type of lamp, but it has obvious advantages and is recommended by those who have used it.

CHOICE OF MICROSCOPE

For photomicrographs at the ordinary laboratory magnifications, any good microscope will do, provided that the tube is dead-black inside to prevent reflections; for low-power photomicrography, however, an instrument with a tube of larger caliber is necessary in order to accommodate the true image, which is too long to "fit" into the ordinary microscope body. For very low power pictures special objectives, closely resembling ordinary photographic lenses, are needed. These have a long working-distance and their use necessitates the removal of the substage condenser in order to secure even lighting. By very low power, diameters of x 10, x 20 are meant. For higher power photomicrographs the best apochromatic objectives are preferable, for one must get the cleanest-cut image possible. Two types of oculars may be employed, those suitable for use with apochromatic objectives in routine microscopy, and special "projecting oculars," which have an adjusting sleeve that enables one to secure a flatter field than that obtained by the others, but one that is restricted and smaller. If one be using the full capacity of the plate-holder, a plate that fills it entirely, the projecting ocular is best, but requires a long bellows extension to get the desired magnification. If one be using "kits," however, that is to say wooden frames that enable one to fit into the plate-holder plates that are smaller than it is, there is a considerable margin of wood at the periphery of the field. In this case it is better to use compensating oculars and project an image sufficiently larger than the photographic plate to place the hazy, peripheral portion of the field beyond its limits. That portion of the field falling on the plate will then be flat and clear, and the magnification greater for a given bellows extension. To illustrate: one uses a 5 x 7″ plate in a 9.5 x 12″ holder, the clear image fills a space 5 x 7 inches, the hazy portion falling on the wood of the "kit." Fields may be rendered still flatter by removing the upper lens assembly of the aplanatic condenser and using its lower lens alone.

CHOICE OF SHUTTER

Any good photographic shutter of the lens-plane type with speeds from $\frac{1}{50}$ to one or more seconds and a "bulb" and "time" mechanism is suitable. It should be large enough to accommodate the entire light-beam entering the substage condenser. It is possible to dispense with a shutter, using a card, or one's hand, or the light-switch, but this is applicable only to long exposures which can be reckoned in seconds, rather than fractions thereof.

CHOICE OF MICROSCOPIC SECTION

The subject to be photographed must be very carefully selected, the thinnest possible paraffin section being chosen by preference. The field to be taken should be picked out under one's own microscope in the quiet of the laboratory, the best grouping of its salient features decided upon, and the place marked on the cover-slip so that it may be found easily when it is to be photographed. The best marker is of the type of Zeiss "Objectmarkierer," a tiny diamond chip mounted eccentrically on a revolving post that

takes the place of one of the objectives on the nose-piece. One selects the field and turns in the marker, just as one would turn in another objective, lowers it until the diamond is in firm contact with the cover-slip, and rotates the post. The diamond scratches a minute circle (which may be increased in diameter by means of an adjusting screw) around the field. These circles can be found best, when looking for them on the ground-glass finder of the camera, by cutting down the light and focussing on the cover-slip. Another way to identify fields is to mark them with a pen and an ink composed of methyl violet, 0.1 gm., best white shellac, 10 gm., and 95 per cent alcohol, 20 to 25 c.c. (Mallory and Wright, 1918). A circle may be drawn, or a dot made near the field. This shellac is insoluble in water and xylol, so that the slides thus marked may be cleaned of cedar-oil without removing the identification marks, which are easily removed with alcohol if so desired.

The thinness of the section to be photographed must be stressed. Thick sections are difficult to focus in one plane and a photomicrograph is always marred if too many details are out of focus, indeed it is well-nigh impossible to get everything into sharp relief under the most favorable circumstances and a five micron section will come much closer to the ideal than a thicker one. It must be remembered that one can photograph no more than can be seen in the section, a perfect photomicrograph requires a perfect section and stain. Knife scratches, blemishes due to precipitates, or other artifacts stand out with horrible distinctness in a photograph, where the elements of color and motion are lacking and technical blunders are emphasized and faithfully reproduced.

CHOICE OF STAINS

This is of equal importance with the choice of the section. Mallory and Leavitt (1922) give a list of stains to which little can be added. For common use they recommend the eosin-methylene-blue stain after Zenker fixation, or the Giemsa or other modification of the Romanowsky method. These give a wealth of detail and the color values of the nuclear elements are most faithfully reproduced. Hematoxylin-eosin has two disadvantages: the nuclear detail is rather meager (although more contrast is obtained) and the eosin may be dense enough to obscure the nucleus. It is a very good stain for this purpose if the eosin be not too intense and the cytoplasmic elements comparatively delicate. If one desire an accentuation of cytoplasmic density, it is unexcelled. Phosphotungstic acid hematoxylin is splendid for mitotic figures and fibrillae, as Mallory and Leavitt point out. It has the habit of turning from deep navy-blue to claret-red after a few minutes under the action of concentrated light, but this appears to make no difference in the photographs. One must guard against "chalky" effects when photographing phosphotungstic acid hematoxylin sections, for the nuclei and fibrils stain deeply, the cytoplasm very weakly and the result is like a line-drawing without shading, and therefore apt to be harsh. This can be overcome by correct timing of the exposure (longer, rather than short) and by using softer paper for printing from the negatives. Almost any stain may be used in photomicrography if

the ray filter combination employed be correct, but the above are the best for general use.

CHOICE OF MAGNIFICATION

One should determine the magnification that best shows the details to be emphasized in the picture, and then find out how it may best be secured. The photomicrographic illustrations of the average medical article are usually taken at far too low magnification and therefore fail to give sufficient detail to illustrate the text. It is, indeed easier to take high-power photographs, for the lighting is apt to be more even and the detail need not be so clean-cut if it be displayed on a large enough scale. Before attempting photomi-

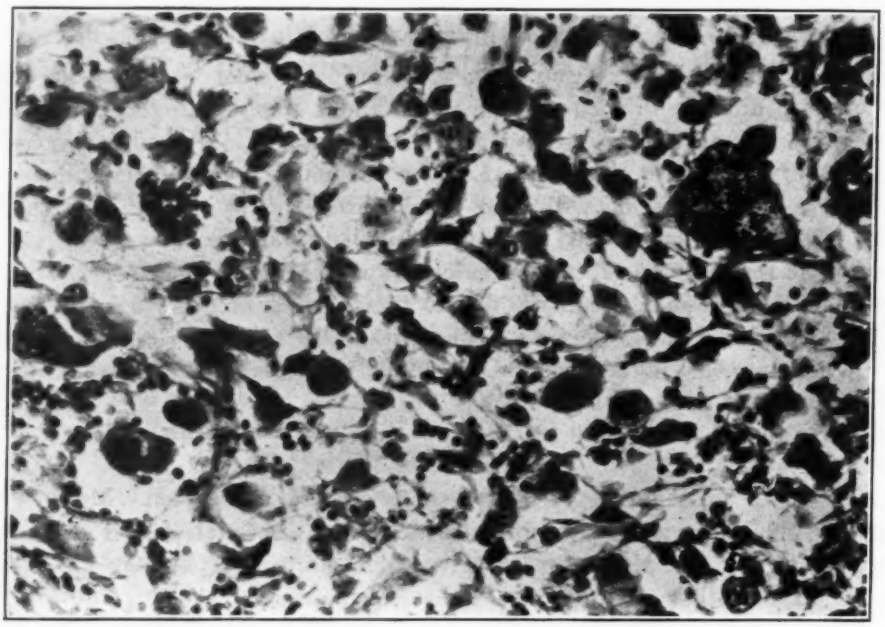

Fig. 1.—Obj. 16 mm. Oc. x 15, bellows 54 cm. Filters Wratton M, B + G; time 2 sec. Plate Standard Orthonon 5 x 7 inches.

crography one should always construct a table of magnifications, as explained in Petrunkevitch's article and in the Kodak pamphlet. A given combination of ocular and objective is put onto the microscope and focussed on a stage micrometer. The image cast with the bellows as nearly collapsed and as fully extended as possible should be measured on the ground-glass and the magnification calculated for each position. The intervening extensions will give magnifications that vary directly with the length of the bellows. These may be calculated, or worked out by actual trial, with the bellows so adjusted as to give one a series of round-number magnifications x50, x150, x200, etc., which should be recorded in a table. One proceeds in this manner with all the lens combinations until a complete table is constructed that will show a consecutive series of magnifications from, say fifty to fifteen hundred diameters, and the combination of lenses and bellows extension needed to procure

them. This table should then be posted on the wall near the apparatus for ready reference.

With thin sections it is always true that the best and clearest images will be obtained with the weaker oculars and the least possible bellows extension. This is because the true image is produced by the objective, its clearness depending upon the resolving power of that lens. The ocular merely enlarges this image and the bellows extension continues the process, but the resolving power of the objective remains unchanged. The more powerful objectives project a larger image and their resolving power is adjusted to that image, so that it is not only larger but remains clean-cut. Therefore it is better, in order to get a larger image, to increase the power of objective employed; rather than to enlarge and disperse the picture projected by a low power objective by using a higher ocular and a longer bellows. The longer the bellows and the stronger the ocular, the less distinct is the image.

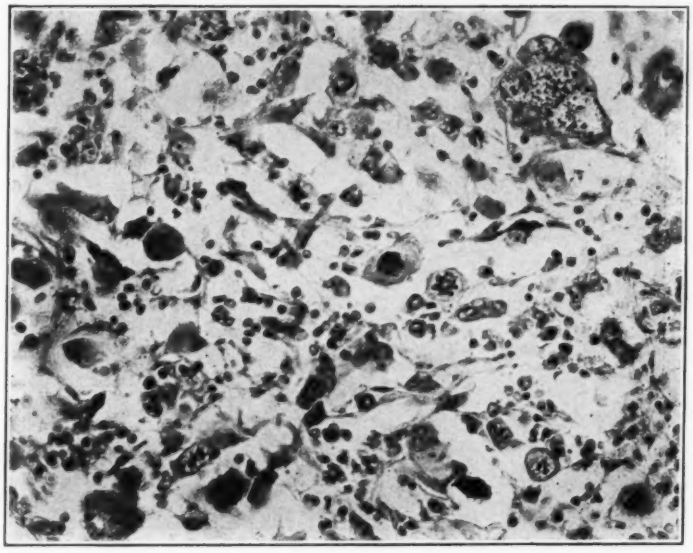

Fig. 2.—Obj. 4 mm. Oc. x 7.5, bellows 24 cm. Exposure 1 second, otherwise like Fig. 1.

Fig. 1 was taken with a 16 mm. objective, a x15 ocular, and a bellows extension of 54 cm. Compare it with Fig. 2 which was taken with a 4 mm. objective, a x7.5 ocular, and a bellows extension of 24 cm. There is less detail in the former, with a larger field; more detail in the latter, with a smaller field, limited by a circle of unexposed plate that has been trimmed off the print. The second picture is undoubtedly superior to the first. If the section were thick, because of tough material or poor technic, the first combination would be preferable to the second as it gains in depth of focus whilst losing in detail.

CHOICE OF FILTERS

This subject is thoroughly discussed in Petrunkevitch's article, as applied to Cramer filters; and in the pamphlet of the Eastman Kodak Company, in connection with the Wratton series. These colored gelatine discs, mounted

between glass sheets, are useful in many ways and for various reasons. It is much easier to employ them than it is to make up fluids to fill the more cumbersome cells of the horizontal outfits and they are almost indispensable in connection with vertical cameras, where they are simply laid on the limbs of the microscope beneath the substage. Tables of the spectral areas intercepted by these filters are given in both of the articles mentioned, when used singly and in combinations of two so that one may choose the proper combination for a given stain.

The fundamental fact to remember is this: to obtain contrast, use a filter, or combination of filters of a color complementary to that of the stained structure to be brought out; to obtain detail use the same color. Contrast is usually the desideratum and the common stains already men-

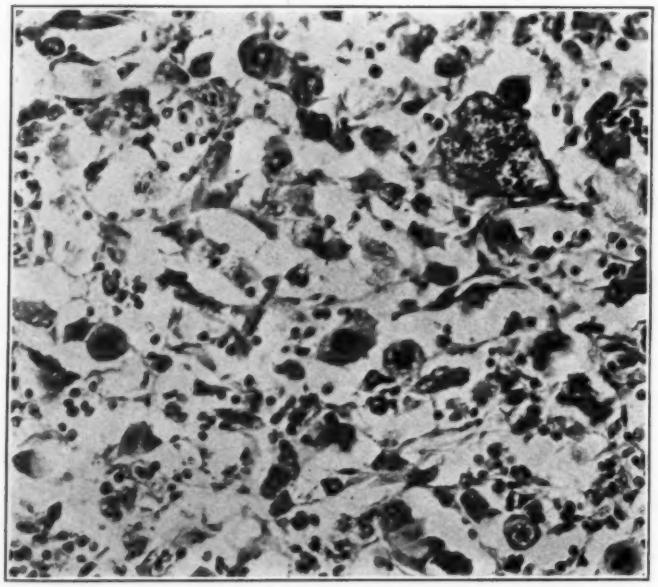

Fig. 3.—Same combination except plate, which is Wratton M Panchromatic.

tioned will therefore require a yellowish-green filter, for they all are more or less violet and red. Violet is composed of red and blue; the complementary color of red is green, that of blue is yellow, hence a yellow-green, or greenish yellow combination is generally required to bring out the nuclei, whilst the general greenish tinge is also complementary to the eosin-stained cytoplasm. The fluid filter recommended by Mallory and Leavitt is yellowish-green and is made up as follows: copper sulphate—175 gm., potassium bichromate—17 gm., sulphuric acid—2 c.c., water to make 500 c.c. Other fluid filter solutions will be found in the Zeiss catalogue of photomicrographic apparatus. It is far cheaper to use the fluid cell than to buy a set of gelatine filters, but the expense of the latter can be reduced to one-tenth by purchasing them unmounted and mounting them between two large neurologic cover-slips bound at the edges with black gummed paper or adhesive tape. They are more practical, cannot leak fluid, are light and portable, can be

varied by forming combinations, and make a wider choice of microscopic stains possible.

As an example of the use of these filters take Figs. 3 and 4. Both pictures were taken on the same sort of plate, the Wratton Panchromatic, the exposures were equivalent, the field was the same in the same microscopic section. In photographing Fig. 3, Wratton filters B + G were used, in Fig. 4 A + D. B + G gives a yellowish-green light, A + D a deep red. The delicacy of detail is seen in the latter, whilst the former gives the best general picture because of the more prominent cytoplasm. The red light has "washed-out" the eosin and accentuated the greener blues of the Giemsa stain, the greenish light has accentuated the reds and purples of the picture.

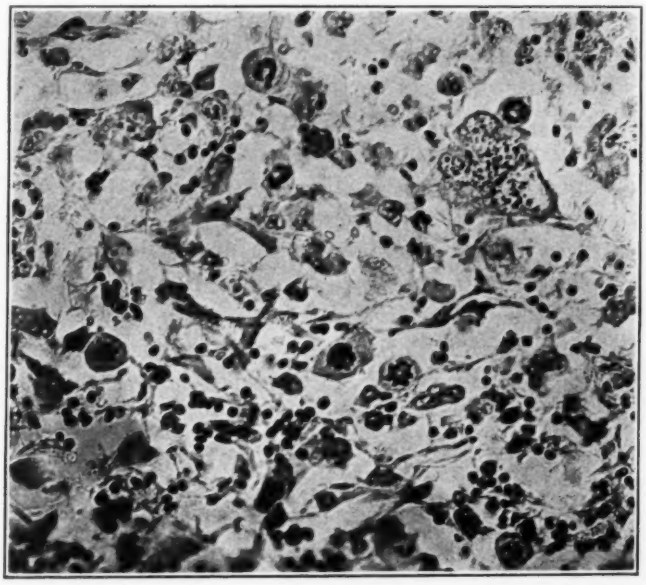

Fig. 4.—Same as Fig. 3 except for filters which are A + D and exposure of 2 seconds.

Fig. 4 could not be taken on any but a panchromatic plate because of the red light employed.

An accurate knowledge of wave-lengths in the spectrum is not essential, all one must know is that if the light coming to a certain stained object be filtered through a screen of a color complementary to that of the stain, the resulting image will be nearly black. For this reason complementary filters are used to bring out a monochromatic, black and white picture, which is technically known as "contrasty." If one is photographing a stain for the first time, the table of spectral wave-lengths may be neglected and various filters, or combinations thereof tried until one succeeds in getting an image on the ground-glass that is as nearly achromatic (black and white) as possible. This will be the proper combination to use. Sometimes this results in cutting down the light through the microscope until an inordinately long exposure is necessary, or until focussing is difficult on account of lack of light. It is

then advisable to substitute some filter combination that is less effective chromatically, but more so actinically. For example, the Wratton combination G + H is often the best on the ground glass, but requires too long an exposure with a given brand of plate: B + G may be substituted, or the brand of plate changed to one more sensitive to that light. B + G in the Wratton, or 5 + 7 in the Cramer series will be found the most suitable for general use.

CHOICE OF PHOTOGRAPHIC PLATES

Mallory and Leavit, after long experience, recommend Cramer Iso Slow plates for general use, Petrunkevitch prefers Eastman Standard Orthonon, or Cramer Iso Medium or Rapid. The Orthonons are satisfactory for the reason that the double coating makes a white background during development, upon which the image comes out in bold relief and is never submerged by a general blackening, and because the blacks obtained are rich and contrasty. The Iso plates tend to turn more generally dark during development and the image is not so well seen after a certain point is reached, but the plates give excellent negatives if development is carried out according to instructions.

At this point something must be said concerning the photographic plate in photomicrography as compared with photomacrography, or ordinary daylight photography. In the former we are dealing with filtered monochromatic light, in the latter with the entire spectrum of daylight. The behavior of a given brand of plate toward the two types of light may be quite different. Some plates are very sensitive in the green section of the spectrum, such as the Cramer Iso series, whilst others are proportionately sensitive to blues, yellows, or other particular colors. Petrunkevitch gives tables of factors for plates of different makes based upon their daylight speed and filter speed. As an illustration of the difference in behavior of different brands of plate with sunlight and with monochromatic light, take the Standard Orthonon and the Cramer Iso Medium: the former is twice as rapid as the latter in daylight, but four times slower in the green, or about the same speed as the Cramer Iso Slow. This is discussed at some length in the article just quoted. Petrunkevitch's R-P factors, as given on p. 302 of that paper will be found helpful in gauging the exposure-time for different filter combinations, but it is best to make out a table for yourself; for the factors do not apply to Wratton filters, which differ somewhat from the Cramers, and are also altered if one use the yellower light of a tungsten lamp.

It has just been said that the most serviceable filter for routine photomicrography is of a yellowish-green color, theoretically it would seem useless to bother about panchromatic plates unless using red light, to which they are peculiarly sensitive. A glance at Fig. 3, which was taken in green light, will show that the Wratton M Panchromatic plate gives excellent results with the B + G combination. The development of these plates is not as formidable as one would suppose, the "safe-light" of pale green gives very little light to see by until one's eyes become accustomed to it, after which it is quite adequate. Panchromatic plates certainly deserve more use in this line of photography, for they enable one to employ almost any color combination in the filters and give great latitude in the choice of the microscopic stain.

CHOICE OF EXPOSURE TIMES

This can best be decided upon by making trial exposures with the chosen combination of filter, lenses, and bellows extension, after working out an

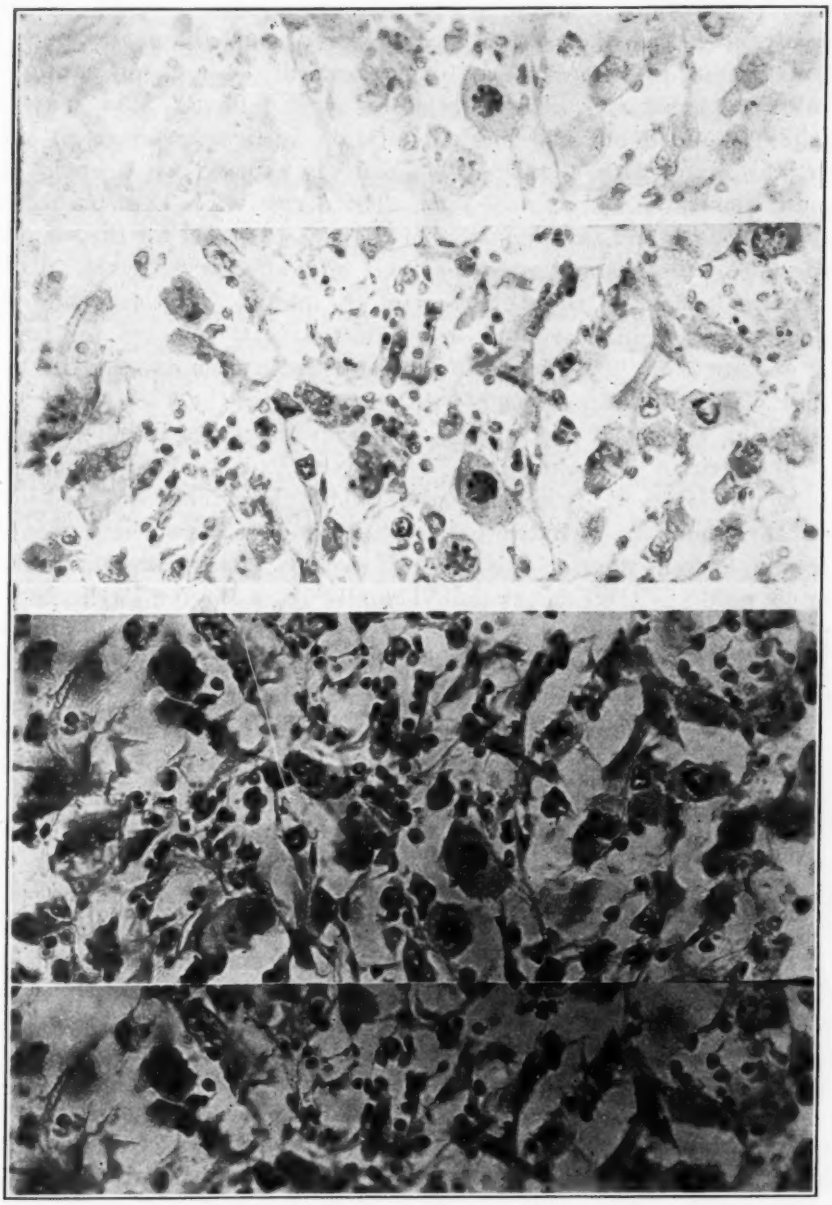

Fig. 5.—Trial plate, exposures range 1/2, 1, 2, and 4 seconds per strip. Combination of filters, lenses, etc., the same as in Fig. 2.

approximate exposure time with the aid of the various factors given in Petrunkevitch's article and the Kodak pamphlet. Sections vary so in density, that trial exposures are almost a necessity. There is an ingenious German

plate-holder in which the plate can be slid along in a carrier behind a mask with a central slit, and thus exposed a strip at a time. Fig. 5 shows the trial plate for Fig. 2; the segments were exposed ½, 1, 2, and 4 seconds respectively, and the second strip demonstrates that one second is the correct time. A method applicable to ordinary plate-holders is somewhat similar but has the disadvantage that the camera may be jarred while the dark-slide is being manipulated and the superimposed exposures will not register, causing blurring of the photograph. The procedure is as follows: The plate-holder dark-slide is pulled out and marked off into vertical quarters on the side toward the plate, with a ruler. The plate is exposed as a whole for the minimum time estimated as necessary. The dark-slide is then pushed to the first quarter mark and three-quarters of the plate exposed for the same length of time. The first exposure equals "x" and affects the whole plate, the second "2x," affecting three-quarters of the plate. The dark slide is then pushed in another quarter-mark and half the plate exposed for twice the initial exposure, or "2x"; half the plate will have been exposed x + x + 2x, or "4x." The procedure is completed by pushing in the slide to the ¾ mark and exposing the remaining quarter-strip four times the initial exposure, or "4x," the strip representing "8x." Applying this to Fig. 5, had it been taken by this method, the whole plate would have been exposed one-half second, three-quarters of it two half-seconds, half of it two half-seconds plus one second, and one quarter of it two half-seconds + one second + two seconds; the totals would be the same as those actually given the four strips in making the plate for this figure.

The type of negative desired is usually a bold black and white, or contrasty one, for we are seeking contrast in photomicrography, as the prints are usually intended for half-tone reproduction, in which the whites are gray and the contrast correspondingly reduced. For some types of subject the negatives should be thinner than for others, a stain for mitotic figures and fibrillae may be photographed so as to produce a rich negative, whilst a diffuse stain may need slight underexposure to bring out contrasts. If the magnification be changed by extending the bellows, or substituting another ocular, the exposure will vary directly with the square of the magnification, but if the objective be changed the numerical aperture figures in the calculation and complicates it, whilst the use of oil-immersion changes it altogether. It is usually easier to work out the new exposure time by trials than to try to calculate it after changing the objective.

CHOICE OF DEVELOPER

For beginners, and in warm weather, the best method of developing is the tank process, and the best developer the Eastman Tank Pyro Powders. All solutions should be chilled, or warmed, to a definite temperature, the developer diluted to the proper strength, and the plates developed according to the table in the directions. If these be followed the results are perfect if the exposure has been correct. The only drawback is that this method takes time and one cannot detect overexposures during the process and restrain the development.

For tray developer the old-fashioned pyrogallol developers are the best, as they give rich, warm negatives and one has a good deal of latitude in exposure, the time may vary a good deal without affecting the results materially. The pyrogallol-soda developer is a favorite and has but one defect, that of staining the fingers. It is composed of two solutions: A. Water—1 liter, pyrogallol—20 gm., potassium metabisulphite—2.5 gm.; B. Water—1 liter, anhydrous sodium sulphite—100 gm., anhydrous sodium carbonate—40 gm. A and B are mixed in equal parts and 10-12 drops of 10 per cent sodium bromide added to each 120 c.c.

The pyro-acetone developer recommended by Petrunkevitch gives excellent results and does not stain the fingers. It is made up of two solutions: A. Water—1 liter, oxalic acid—2 gm., pyrogallol—60 gm.; B. Water—1 liter, anhydrous sodium sulphite—120 gm. Fifteen parts of A, thirty of B, six of acetone, and one-hundred-twenty of water are mixed and used fresh. Develop for about six minutes at 18.5° C. (65.5° F.); the image should appear in about fifteen seconds in correctly-timed negatives.

There is no choice in fixatives, an acid hypo bath is best as it hardens the gelatine coating on the plates and prevents frilling and scratching; it is imperative to use it with the double-coated Orthonon plates. Chrome alum hardener may be used if preferred.

CHOICE OF PRINTING PAPERS

There is no use in experimenting with matt papers, or with the artistic, soft effects admired in gross photography—one must get the maximum detail, which is obtained only with glossy papers. As a rule a glossy contrast "gaslight" paper is the best, such as Azo F No. 4 or Cyko Contrast. Normal grades give too gray a background for half-tone reproduction, as shown in Fig. 6 which was printed from the same negative as Fig. 3, but on Azo F No. 2 instead of F No. 4. One must try for dead white backgrounds and black details. Any good "M-Q" developing powder, or metol hydrochinone developer will give the desired results. It will be found advisable to add a few drops of 10 per cent potassium bromide to the developer if the whites are at all gray.

The prints should be exposed for the proper time, developed to the depth desired and, after fixing and thoroughly washing, rolled down on a ferrotype plate with a squeegee, following this up by a rerolling under a sheet of blotting paper to remove all surplus water. The prints should curl off the plate spontaneously, when dry, or be pulled off gently after raising one corner with a penknife. The ferrotype plate should be dressed once or twice a season with a little paraffin in benzol, or beeswax in turpentine. Usually, however, a thorough washing with hot water and a polishing with a soft rag, followed up with absorbent cotton, will leave a polished surface to which the prints will not adhere too firmly. If they stick, they should be soaked off and the process repeated, after dressing and polishing the plate. Sometimes it is well to soak the prints in 25 per cent alcohol, as recommended by Mallory and Leavitt, before rolling them down. This insures more even ad-

hesion while drying and prevents dull spots appearing. A glossy surface is desirable not only because of the increased detail in the print, but because it is the best for reproducing in half-tone plates. Dull, spotted prints will reproduce as dull, spotted half-tones.

CHOICE IN MOUNTING

It is best to mount the prints separately on thin cardboard by means of a process that can be reversed and the prints remounted by the publisher. The best way of doing this is to take a light grade of cardboad, cut it into cards slightly larger than the prints, and mount the latter on these by means of Eastman's mounting tissue. This is in the form of dry sheets of gelatine, resembling isinglass in its appearance. A sheet is tacked to the back of the print by touching it here and there with a hot spatula, the two laid on the

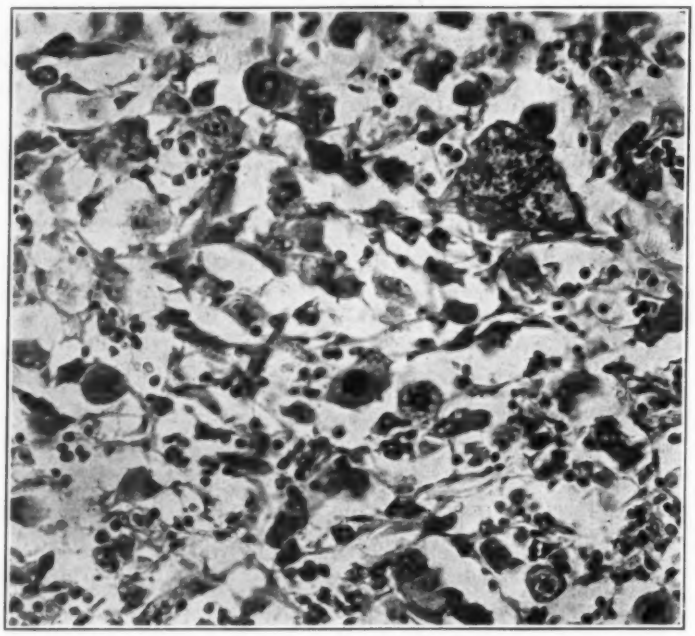

Fig. 6.—The same as Fig. 3 but printed on Azo F No. 2, a normal, glossy paper. Note the increased grayness and softness of the effect, which is not as desirable as the more contrasty print shown in Fig. 3.

mount and the tissue melted with a hot flat-iron, or a hot-press made for the purpose may be utilized. This method makes it possible to remove the prints and remount them by heating them and pulling them off whilst hot. It is so much superior to any other method of using glues or pastes, that there is no comparison—the prints are flat, glossy, unstained, and firmly incorporated with the mount. After mounting, the print may be trimmed to the desired size. A good deal may be done to enhance the value of the prints by trimming off hazy borders, rearranging salient features of the photograph by centering, and deleting unessential portions of the field. Such mounted prints can be mailed in a manuscript and do not take up much space, at the same time being stiff enough not to bend easily in transit. They may be

mounted *in toto* on a larger backing by the electrographer, for the cardboard is thin and will lie flat on a heavier mount.

CONCLUSION—ELEMENTS THAT LEAVE NO CHOICE

Having considered the elements of choice, let us briefly review those that leave none. Granted that the apparatus be suitable for the production of photomicrographs, what precautions must be taken to prevent spoiling these in the making? In the first place the section to be protographed must be perfectly cut and stained and the field selected in advance. Whatever the apparatus, the source of light must be centered and focussed on the substage condenser. If an electric arc, the carbons must be adjusted to give the steadiest and brightest crater and the poles must not be reversed. In the horizontal apparatus, the various riders on the optical bench must be at the proper relative distances from lamp and microscope, and the lenses and diaphragms that they carry must be centered around the beam of light.

The filters must be in place when the exposure is made, one often forgets to replace them after focussing and then wonders why the plate is so badly overexposed. The microscope must be aligned with the axis of the beam from the lamp, the condenser centered by using its nearly closed diaphragm as a marker. In the horizontal outfits the objectives are usually carried in adjustable sliding holders which have two centering screws, and the objective must be so centered that no prismatic crescent will show at one margin of the field. Furthermore, the collar into which this holder slides, is also centerable, as it carries the photographic lens when it is used for low-power work, so that it must also be adjusted until a beam of light coming through the substage diaphragm fills its aperture evenly, not overlapping one margin or another. In the small vertical outfits the microscope need merely be centered as a whole. The precautions necessary in adjusting the bellows are practically those essential to ordinary photography. The extension must be set at the desired point and the aperture fit snugly into the sleeve over the ocular of the microscope to prevent stray light from entering and fogging the plate. The dark-slide must always be pulled out of the plate-holder before making an exposure and replaced after it is finished. Careful focussing is indispensable, one should always wait a little after focussing to be sure that the slide does not settle in its clips, or the jaws of the mechanical stage, thus getting out of focus. The bellows may settle a little if not firmly screwed into place, particularly in the vertical outfits.

Dark-room "dont's" will be found in any elementary book on photography. Perhaps the most important are: don't use old, or dirty solutions and keep everything as clean as possible, avoiding dust and scratching. Don't handle the negatives any more than is absolutely necessary. One is not finished with a photograph until it has been mounted, labeled, and mailed to the publisher, and any carelessness after the picture has been taken is as ruinous as a fault in the apparatus. Old plates and paper may, or may not give trouble, it is a gamble as to the outcome of a photograph taken after the manufacturers' time-limit on plate or paper has expired.

Photomicrography may be summed up, in closing, as an exercise in scrupulous care and a drill in going through certain almost mechanical motions. After a time, the photomicrographer will find his attention focussed on the choice of the slide, the stain, the ray-filter combination, the plate, and the paper to be used, with a passing thought to the exposure time. All the other steps will have become second nature, with occasional ludicrous lapses into absent-mindedness. The all-important thing to bear constantly in mind is cleanliness and neatness—one cannot make a good photomicrograph without exercising these two essential attributes.

EXPLANATION OF FIGURES

All these photomicrographs were taken on a large Zeiss horizontal apparatus, fitted with automatic 5 amp. right-angle electric arc; one collector lens; lower lens of Zeiss aplanat condenser; Zeiss apochromatic objective and compensating ocular; and developed with pyro-acetone developer. They were printed on Azo F No. 4 Glossy paper, excepting Fig. 6, which was Azo F No. 2; and developed with M-Q powder. The section is a malignant tumor stained by the Giemsa's method. Magnification in all figures about x400 diameters.

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A SIMPLE METHOD OF CONTROLLING KYMOGRAPH RATE*

By P. D. Lamson

IN simple kymographs it is often difficult to obtain slow speeds without the use of very large fans and often with even these, a slow enough rate cannot be obtained. It was found that by immersing the fan in fluids of different viscosity and to various depths, gradual variations in rate could be obtained, down to an extremely slow movement of the fan. The fast rates are best controlled by the air fans and the slow rates are conveniently obtained by using oils of different viscosities for immersion of the fan. The depth of the oil, when a very slow rate is desired, is not as important as the viscosity, as the oil itself takes on motion.

The apparatus can be easily made by cutting a central hole in the bottom

*From the Pharmacological Department, Johns Hopkins University.
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of a tin can, and soldering in a short brass tube. It is well to solder three small legs onto the bottom of the can to raise it above the fan brake. For those not equipped to turn a fan stem on a lathe to fit the kymograph spindle, cutting off the stem of one of the air fans and soldering this to a piece of

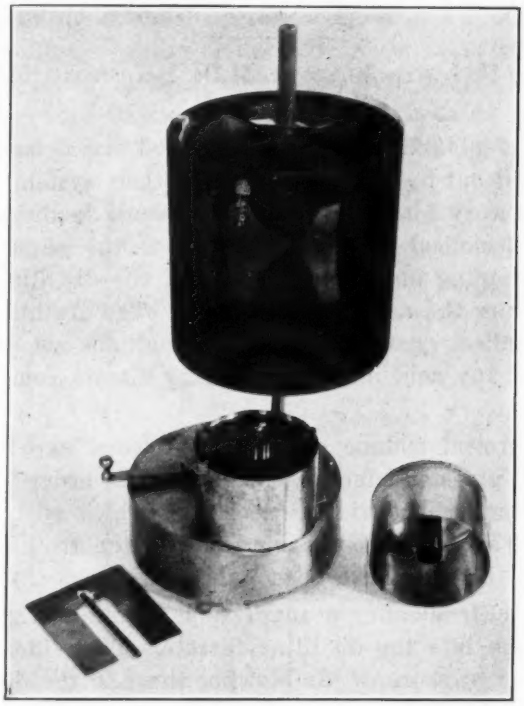

Fig. 1.

brass cut as the fan shown in the figure, will be found a convenient way of making a stem which will fit.

An apparatus of this type made for use with the student's Harvard kymograph is shown in the figure.

A TECHNICAL IMPROVEMENT IN THE DETERMINATION OF BLOOD UREA BY THE FOLIN-WU METHOD*

By S. Lloyd Johnson, M.D., Baltimore, Md.

SEVERAL technical difficulties have attended the determination of blood urea as worked out by Folin and Wu,[1] in their system of blood analysis. Granting a satisfactory filtrate, an active ferment, facilities and experience, there still remain technical difficulties inherent in the original method. Chief of these is the bumping and frothing over of the distilling filtrate and the back aspiration from the acid-receiving tube. The frothing over introduces no serious error, unless gross in amount, and perhaps not even then,[6] but the back aspiration of any acid into the distilling filtrate completely invalidates the result.

With the improved technic acquired by special experience the original method, no doubt, gives satisfactory results; but in order to make it safe for the average worker some device is necessary which will prevent the back aspiration of acid and will, if possible, obviate the frothing. A number of suggestions along this line have been published:

Boggs and McElroy[2] offer a suggestion for preventing back aspiration of the acid solution into the distilling filtrate. Their idea is to maintain a continuous positive pressure of air blowing through the delivery tube. This is accomplished by forcing a current of air into the delivery tube just beyond the bend of its distal arm. This continuous positive current of air which is kept bubbling through the acid-receiving solution prevents, they claim, back aspiration of the solution.

An objection to this suggestion is the necessity of additional apparatus for generating the current of air.

Moore and Jones[3] make use of positive air pressure by use of a tube drawn out to a capillary point and carried down under the surface of the distilling solution. A constant current of air is thus kept bubbling through the distilling solution. This prevents the bumping and frothing and by maintaining positive pressure in the system, prevents the back suction of the acid solution. The air pressure is maintained by use of an inflated rubber bag. The tip of the capillary tubing is carried down to within 0.5 cm. of the bottom of the distilling tube. (This idea is utilized also in doing non-protein nitrogen determinations by the Folin-Wu method.) By this method they claim to be able to increase or decrease the size of their flame without technical difficulties or accidents.

G. E. Youngburg[4] makes his distillations with an open delivery tube,

*From the Department of Medicine of the University of Maryland, and the Clinical Pathological Laboratory of the University Hospital, Baltimore.
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that is, the delivery tubing is carried down to within 1 to 2 cm. of the surface of the receiving acid, but is not carried under the solution. He claims that the ammonia so delivered is not lost but is taken up by the acid, providing the receiving solution is kept below room temperature. This is done by half immersing the receiving tube in a beaker of cold water. Needless to say, there can be no reverse aspiration by such a method. Comparative figures are given supporting the claim that no ammonia is lost by this *open,* as compared to the *closed* method, and while their figures are reassuring, one cannot help feeling that some ammonia must at times be lost, and especially so, if the distillation is a little too rapid. A subsidiary suggestion is the use

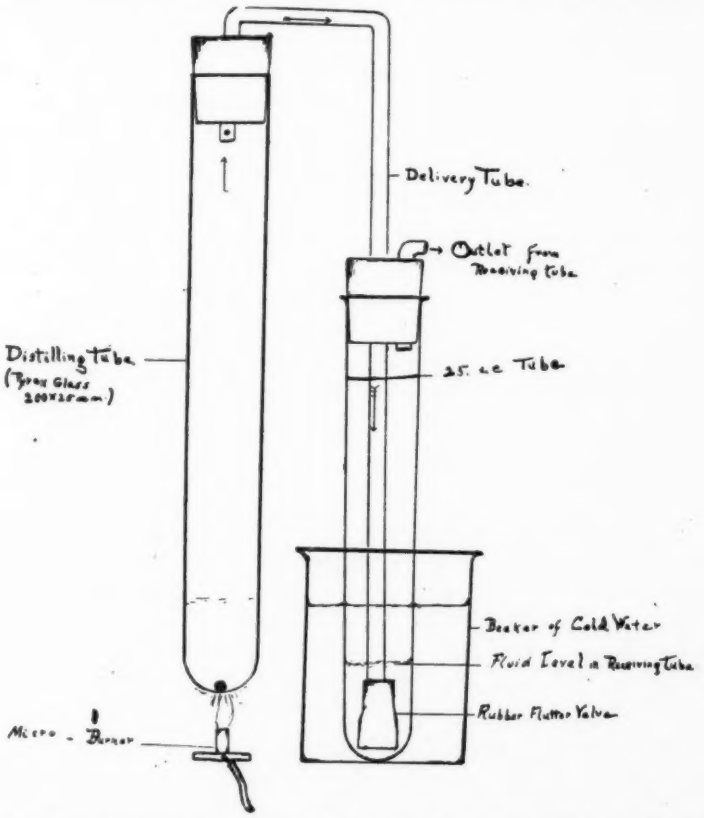

Fig. 1.—Folin-Wu urea distilling apparatus with flutter valve attachment. (3/8 actual size.)

of American Oil (Parke, Davis & Co.) instead of the ordinary paraffin oil. The cheaper oil is distilled over to a certain extent and the clouding which results interferes with accurate color comparisons after "Nesslerization."

Watson and White[5] encountered frothing in distilling the filtrate and attempt to overcome it by an especially devised conduction tube. They make use of a 25 c.c. bulb pipette bent in a special manner. The apparatus is illustrated in their article.

Feinblatt[6] eliminates the usual distillation completely and after proper ferment splitting of the urea molecule "Nesslerizes" in the original tube. Parallel tests made *with* and *without* distillation give him results with no

greater differences than could be accounted for by individual variations in reading the colorimeter. Practically, good results may be obtainable, but from a theoretical standpoint one doubts the advisability of such abbreviation in all cases. In those cases where we have tried this suggestion it has checked up fairly closely with the distillation methods.

The following modification of the Folin-Wu method is offered, only because it seems to possess certain advantages of simplicity and practicability over those described: We make use of the original Folin-Wu method of determining blood urea. We thus use the apparatus which most laboratories doing work already have. The only additional apparatus required is a small one-way rubber flutter valve. (The flutter valve found on the gas masks used by the soldiers is what we have in mind, only miniature in size.) Such small flutter valves are found in the stem of toy rubber balloons and are used for inflation. They permit an ingress of air but effectually prevent its escape. The flutter valve used by us was taken from the stem of a toy balloon purchased for a dime at the five and ten cent shop. This flutter valve is merely a short length of rubber tubing flattened at one end and held more or less rigidly, but with sufficient give to it to permit air to pass one way only, and clamping itself off effectively when any pressure (air or fluid) begins flow in the reverse direction.

Consulting the accompanying illustration it will be seen that this rubber flutter valve is slipped on to the distal end of the delivery tube and allowed to deliver the distilling ammonia to the bottom of the receiving acid solution. If this rubber valve is ordinarily good there will be no back aspiration of the acid, even under the most unusual conditions. The flame can be increased or decreased with indifference as to results. The greater the tendency to a vacuum in the system, the tighter the valve holds. Occasionally the fluid may rise up a short distance into the delivery tube but never has it gone over into the distilling tube. To insure closure of the flutter valve, an ordinary spring clothes-pin is clamped onto the valve end when it is not being used. (Many substitutes may be found for the clothes-pin, achieving the same end, as well, or even better.) This is not necessary but is good practice.

In addition to the above suggestion, it is good practice to follow the suggestion of Youngburg[4] and keep the receiving tube below room temperature by immersion in a beaker of cold water. This further insures absorption of the ammonia delivered.

Our experience also favors the use of the pyrex ignition tube as recommended by Folin and Wu, originally, in place of the lighter glass substitutes. The heavier glass maintains a more constant temperature in the distilling tube with less sudden eruptions of the distilling filtrate. The longer the distilling tube, the cooler the top, and the more likelihood of the subsidence of the frothing before escape. Little or no frothing over will be experienced if these precautions are taken.

SUMMARY

A simple modification of the blood urea method of Folin and Wu is described which has been found to do away with reverse aspiration of the filtrate.

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A NOTE ON CERTAIN LIMITATIONS OF THE USE OF SOLID MEDIA FOR THE DETECTION OF GAS PRODUCTION BY BACTERIA*

BY L. O. DUTTON, MEMPHIS, TENN.

WHILE studying the fermentation reactions of certain organisms that were known to produce gas from given sugars an occasional tube was encountered that gave a negative result. In this work solid media was used with dibromcresol as an indicator of acid formation. The acid was readily detected by the indicator but the gas production was either negative or doubtful in a number of tubes. As gas production was known to be a constant finding in studies of the organisms in question, an attempt was made to detect the factor responsible for the unusual findings.

As viscosity of the medium was a likely factor in the mechanics of gas detection, experiments with this in view were undertaken. Tubes of agar agar were prepared with lactose and the indicator. These were sterilized in the autoclave for 15 minutes at 15 pounds pressure. A duplicate set of tubes were prepared and sterilized in the Arnold on three successive days. Each of these sets was then divided into five groups and stored respectively in an air tight humidor, ice box, open at room temperature, at thirty-seven degrees, and at fifty-six degrees. An organism of the colon group was chosen that constantly and vigorously fermented lactose both with the production of acid and gas. A tube from each of the groups was planted with the organism as soon as the sterilization was completed, and a tube was planted on each day thereafter for fifteen days. The plants were all made in the same manner, by streaking the slant of the medium once and then plunging the needle into the butt of the tube. An attempt was made to plant as nearly the same amount of material on each of the tubes as possible. The amount of gas produced was recorded by the number of bubbles in the medium.

*From the Laboratories of the Baptist Memorial Hospital, Memphis, Tennessee.
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Although this is not by any means a quantitative test, it is a criterion that is observed in practice.

From the practical standpoint the results obtained in these tests were very enlightening. In the tubes that were sterilized in the autoclave an average of seven bubbles was obtained in the fresh plants. The tubes stored at room temperature and at the incubator temperatures rapidly diminished in number of bubbles produced and at the end of fifteen days not more than one bubble was obtained in the tubes. The tubes kept in the fifty-six degree box were, of course, too rapidly desiccated to be of value except that after several days no gas bubbles could be detected at all.

Of special interest are the results that were obtained with the tubes that were kept in the ice box. There was a gradual diminishing of the number of bubbles produced in these tubes and at the end of the fifteen days some of the tubes were no better than the tubes that had been kept at room temperature. As the ice box is generally used for the storing of media, it is apparent that here lies a source of error in fermentation studies.

Of very practical importance are the results obtained with the tubes that were stored in the humidor. At the end of the fifteen days there was no more variation from the average number of bubbles in the medium than was obtained with the fresh medium.

In view of these findings it was suggested that it would be more practical to store media in an air-tight container with a pad of moistened cotton or a small amount of water in the bottom.

It was not determined just what was responsible for the failure of the medium to give constant results in the matter of gas production, but it is well known that certain of the delicate pathogens grow only on fresh medium, the moisture content being the apparent factor here also.

In laboratories where fermentation studies are not made on a large scale, it is sometimes difficult to keep freshly made medium at all times without having a large amount of waste in the form of discarded medium, or in repeated labor in the preparation of small quantities of medium.

It is suggested, then, that not only from the standpoint of accuracy of results, but from that of economy also, it is practical to store media in some form of moist chamber, preferably one that can be iced and kept at a low temperature. The moisture, however, is more important than low temperature and should be considered first.

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EDITORIALS

Recent Developments in the Study of Scarlet Fever

THE history of scientific discovery is more often a tale of dogged persistence and repeated attempts in the face of failure than of flashes of genius.

This is well illustrated in the recent studies concerned with the etiology of scarlet fever which bid fair to solve a hitherto baffling problem.

The contagious nature of this disease has long been known and its infectious character long suspected and many investigators have attempted to determine its etiology. The frequency and constancy with which streptococci have been found in the throats of those suffering with the disease is a matter of common knowledge but it has remained for recent studies to demonstrate their probable etiologic relationship.

Tunnicliff[1] in 1920 called attention to experimental work, suggesting that the hemolytic streptococci found in association with scarlet fever formed a

distinct group from the immunologic point of view; but in spite of these findings, Dick and Dick[2] in 1921 were unable to produce experimental scarlet fever in thirty volunteers by the inoculation of pure cultures of hemolytic streptococci isloated from early cases of the disease, thus repeating the experience of previous workers.

The demonstration by various workers, however, of the production of immune bodies, such as agglutinins and complement-fixing bodies, in conjunction with their relatively constant presence in the disease, sufficed to render it impossible to entirely disregard them and the question continued to be made the subject of laboratory studies, until in October, 1923, Dick and Dick[3] reported the occurrence of two cases of scarlet fever experimentally produced by the inoculation of "hemolytic streptococci or some organisms closely associated with them in culture."

In December, 1923, Bristol,[4] in a very thorough résumé of the subject, brought forward a new viewpoint, namely, that scarlet fever might be a manifestation of anaphylaxis to the streptococcus protein, basing his theory upon the numerous points of similarity between drug and serum allergy and the symptomatology of scarlet fever, and demonstrating the feasibility of cutaneous sensitization tests with dried streptococcus protein. His results showed that while from 41 to 61 per cent of normal individuals who had had scarlet fever, and also normal individuals who had never had the disease, gave a positive reaction, those who had the disease gave a uniformly negative reaction, from which he concluded that during the disease there was a state of desensitization. He suggested, therefore, that scarlet fever is a reaction of specific hypersensitiveness to streptococcic protein, and a compound condition involving both a streptococcus infection and, secondarily, a streptococcus protein intoxication in those who are hypersensitive.

In January, 1924, Dick and Dick[5] reporting the results of experiments with the filtrate of the streptococcus culture with which they had previously experimentally produced the disease in two instances, demonstrated the occurrence of a specific cutaneous hypersensitivity and were able to show that it occurred in 41.6 per cent of individuals who had not had the disease; that it was negative during convalescence from the disease; that its action could be inhibited by the coincident presence of convalescent scarlet fever serum, and that when positive prior to the attack, it was negative during convalescence. They thus conclusively demonstrated the specific relation of the test to immunity in scarlet fever.

The practical application of this work to the segregation of susceptible contacts during epidemics of the infection is obvious.

In the same issue of the same Journal the same investigators also reported the fulfilment of Koch's postulates by their culture of a nonmannite fermenting hemolytic streptococcus.

In February, 1924, Dochez and Sherman[7] demonstrated the production of a specific antiserum by the immunization of horses extending over long periods, but were, at the same time, unable to demonstrate appreciable amounts of soluble toxin in the cultures used for immunization, Dick and

Dick[8] in the same issue, proved the occurrence of specific reactions in susceptible individuals when injected with the filtrate of their streptococcus culture and, also, that the reactions could be neutralized and prevented by the blood serum of an individual who had had the disease. Both of these observers concluded that the antisubstance was in the nature of an antitoxin; the presence of large amounts of soluble toxin was not found, however. This apparent inconsistency is explicable in the light of Bristol's theory that it is the streptococcus protein rather than the streptococcus toxin which is of predominant importance.

Blake, Trask, and Lynch[9] found Dochez's antiscarlatinal serum to have district therapeutic and diagnostic value, thus evidencing its specific nature.

Of interest, as indicating that the streptococcus hemolyticus is of etiologic importance because of anaphylactic reactions to its specific protein and thus combining the findings of Bristol with those of other observers, through whose work the relationship of specific streptococci to the disease has been established, is the report by Dabney[10] of four cases of scarlatina apparently manifested only by infection of the mastoid process and the antrum of Highmore.

These patients, nurses, were in attendance on scarlet fever cases and later developed infections of the antrum and of the mastoid from which hemolytic streptococci were isolated in pure culture. These infections were, undoubtedly, contracted from the scarlet fever patients, and yet the nurses, who had never had scarlet fever, failed to develop the usual manifestations of the disease. The problem is summed up by this author as follows: "If the hemolytic streptococci in question, derived from full blown scarlet fever cases, did not cause scarlet fever in these nurses, but merely an infection of the mastoid and antrum, why did they cause scarlatina in those with whom the nurses came into contact and from whom the local infections were derived?" He suggests, as the answer, "Because scarlatina is the result of a sensitization to the streptococcus in its more malignant forms. This would explain its behavior in those who exhibit true scarlatina and in those who have merely local manifestations, such as the four reported here, and those numerous, so-called anomalous cases which show a slight nephritis, sore throat (streptococcic), fever, and little or no rash."

It seems very probable that the future development of the investigations noted may eventually result in the removal of scarlet fever from those diseases generally held, for lack of more definite information, to be due to a filterable virus.

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—*R. A. K.*

The Standardized Wassermann Test

So few vestiges remain of the procedure originally described by Wassermann, Neisser, and Bruck for the serodiagnosis of syphilis that it were better, in the interest of exactitude, that the phrase "complement-fixation test" be used to describe the method in the literature of the future, and the phrase "complement-fixation reaction" to denote the result.

Much has been written of late concerning the necessity for the adoption of a more or less uniform method for the performance of this test in order that the reactions obtained may be more or less uniform and comparable; in spite, however, of the vast amount of work which has been done, a uniform technic has yet to be generally adopted.

There are several obvious reasons for this seeming failure, first and foremost being the fact that the elaboration of a method suitable for a standard is a very different matter from securing its adoption or even its extensive and unbiased trial.

Both the clinician and the serologist—not to mention the patient!—are or should be concerned. The serologist, perhaps, is the more affected for it cannot but be admitted that the introduction, and especially the refinement of the complement-fixation test as tending to make the results more delicate and reliable, has insidiously led to a growing neglect on the part of many clinicians of the careful and detailed clinical study of syphilis with a resultant tendency to rely, more or less, solely upon the complement-fixation reaction as the predominant indication of the presence or absence of the disease.

If, by standardization, the clinician visualizes the evolution of an infallible test, his hopes are doomed to disappointment, for it is not the infection but the response to infection which gives rise to a positive reaction; the technic may be made uniform but the patient cannot be standardized and will react only in accordance with his individual capacity.

The serologist is confronted with a twofold duty in this matter: It must be his aim and endeavor to detect as many cases of syphilis as is possible, and it must also be his endeavor to protect the nonsyphilitic from stigma and unnecessary treatment because of a false positive reaction.

There is no method at present devised without its limitations; none which is perfect; but many which excel, in one particular or another, others in common use. The ideal method to be sought for is that which will com-

bine with the greatest degree of delicacy the highest degree of relative specificity.

It is the duty of the clinician to demand of the serologist a constant endeavor to perfect his technic to approach these requirements, and to subject to fair, impartial, and extensive trial such methods as are advanced upon the basis of careful and well-grounded experimentation. Such investigations must be sufficiently extensive to clearly demonstrate the predominant characteristics of the methods compared or the work cannot be truly informative.

It is the duty of the serologist to be ever on the alert for technical advances; not to adhere slavishly to one method simply because it incorporates favorite modifications, or because he has used it with satisfaction or finds it convenient; and to subject to comparative trial those methods which others have found to be good or which offer some logical or experimental foundation for their claims to excellence—and this search must be unceasing.

It is the serologist's privilege and, indeed, his duty—to demand of the clinician the closest and most extensive clinical cooperation in order that the methods on trial be truly evaluated. Such investigations must be joint investigations or they are worthless. They should be conducted without prejudice and if the method on trial, after sufficient investigation, is found to be superior to that with which it is compared, its adoption should inevitably follow.

Methods must be compared and evaluated solely on the basis of results obtained without regard to time or labor consumed. The clinician or internist cannot evade a thorough physical examination because it is time-consuming, nor the surgeon the adoption of an efficient technic simply because it is laborious. The serologist and syphilologist in a similar situation must take the same stand.

Delicacy is of undoubted importance but specificity must be made paramount and should be the main point at issue.

A method giving only true positives can only be elaborated at the expense of delicacy; an exceedingly delicate technic may be open to the danger of false positive reactions; the solution of the problem lies in a compromise; the middle ground is the safest as long as, in a definite proportion of instances, the reaction is to be interpreted entirely on its face value.

That the standardized method must be a quantitative method is obvious if the test is, as it should be, utilized as a means of following the results of treatment; but whether the quantitative factor depends upon the number of complement units fixed, or upon variations in the doses of serum or antigen is relatively immaterial and dependent upon feasibility and technical considerations. As long as the true nature of syphilitic reagin remains a mystery it will be impossible to accurately measure its amount—nor is this essential. What is aimed at is a means of comparing the relative strength of two reactions regardless of whether or not the yardstick is arbitrary or biologically accurate—as long as the estimations are comparable and relatively free from gross error.

And, finally, when a standardized technic has been evolved and generally adopted, both serologist and clinician must unite in accepting the complement-fixation test as merely *a single phase of the study of syphilis* to be correlated with and interpreted in the light of all the other findings of a particular case.

—*R. A. K.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

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*Medical Science, Abstracts and Reviews**

THIS periodical is an outgrowth of a publication developed during the war for the purpose of keeping the medical officers of the various armies in touch with current advances. It has been continued as a supplement to the Oxford series of publications.

Each number is divided into three sections, the first an editorial review of recent work on some specified subject; the second, reviews of selected journals, and the third or major portion, abstracts of recent contributions classified under special headings such as general medicine, respiratory diseases, metabolism, etc.

Although considerable space is devoted to American periodicals, the abstract service will be of particular value to workers on this continent because of the abundant abstracting of foreign language journals.

Dental Infections†

IN two magnificent volumes—too magnificent perhaps—Price has detailed observations on dental infections which have been made during the past twenty-five years.

Unlike the usual collections of observations these are not merely clinical in the narrow sense. They are based upon painstaking clinical observation, supplemented by bacteriologic and chemical studies, and upon a very great deal of experimental work. They are researches in the best sense of the term.

To the reviewer they represent, as a whole, one of the milestones in dentistry, and will rank historically with Miller's book on the bacteriology of the mouth.

It is a remarkable thing to consider that in the cases with which Dr. Price deals that he has used his material thoroughly. Not only has the oral

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*Medical Science, Abstracts and Reviews. Published for the Medical Research Council by Humphrey Milford. Oxford University Press. Vol. X Number 1, 30 shillings per annum. Printed in England.

†Dental Infections, Oral and Systemic. By Weston A. Price, D.D.S., M.S., F.A.C.D., Chairman Research Section of the American Dental Association. Vol. 1. Researches on Fundamentals of Oral and Systemic Expressions of Dental Infections. Pp. 673, 261 illustrations. Vol. II. Researches on Clinical Expressions of Dental Infections. Pp. 471; 474 illustrations. Cleveland, Ohio: The Penton Publishing Co., 1923.

situation been a matter of study, but the whole physical situation in each individual. Careful clinical histories have been made a matter of record and these have served for studies in inheritance, as it applies to the dental situation. Furthermore use of the material removed has been studied in the same way, and apparently with the same thoroughness as is the material removed from the body by general surgeons in the best hospitals; blood chemical studies and dietetic ones have not been overlooked. It seems that all the best methods of study have been drawn upon to elucidate problems which have been, and which still are, problems largely because of lack of cooperation between the medical and dental professions.

How strongly the head of active interprofessional cooperation appears is indicated in the following paragraphs; "and since patients largely determine the biologic qualities of the organisms involved in dental infections by the culture medium they furnish the bacteria, and since the sufficiently high defense of certain individuals will, under ordinary conditions, protect them from systemic injury resulting from their dental infections; and since the local oral expressions of the dental infection are an indication and a measure of that individual's reaction to the dental infection rather than a measure of that infection, therefore it becomes apparent that the operation that is indicated is an individual factor and concerns the relation of the efficiency of the patient's defense to the attacking power of the dental infection, and, accordingly, operations which are strongly indicated for some individuals are as strongly counterindicated for others. Diagnosis and treatment are therefore shown to be not the simple procedures that we have been accustomed to accept but quite intricate ones in which the clinical history, and a careful clinical study of the case supplemented by accurate laboratory work is as essential as in any other type of clinical case."

The volumes should be a tremendous stimulus to the dental profession. In no publication are the shortcomings of both dental and medical profession so clearly shown. At the same time the whole work is essentially constructive and not destructive.

As time advances these volumes will form one of the medical classics. They are now, with perhaps one or two exceptions, the only great research volumes devoted to the teeth.

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*Problems of Tuberculosis**

A CONTRIBUTION to the public health and administrative features of the control of tuberculosis. The author traces the development of the Anti-Tuberculosis Crusade in England from its beginning in 1840, when George Bodington organized the first sanatorium and advocated open air treatment, through to the present time, when in England tuberculosis work is being carried on by 340 tuberculosis officers in 442 dispensaries. He de-

*Problems in Tuberculosis. By Sir James Kingston Fowler, K.C.V.O., C.M.G., M.A., D.Sc. (Hon.), F.R.C.P. Consulting Physician and Emeritus lecturer on medicine, the Middlesex Hospital, Consulting Physician to the Bromptom Hospital for Consumption and Diseases of the Chest and to King Edward VII. Sanatorium; Member of the Colonial Advisory Medical and Sanitary Committee. Cloth. Pp. 63. Oxford Medical Publications. 1923.

scribes the prevalence of the disease in England and Wales and emphasizes the large proportion of errors in the diagnosis of incipient tuberculosis. He attributes them to four chief sources: (1) lack of adequate incipient case material for instruction in teaching institutions, (2) the increased use of X-ray in diagnosis. This has helped the doctor to evade the responsibility of a diagnosis by placing it on the shoulders of the radiographer. "It is easier to play a pianola than to become a pianist." (3) the radiographer, with rare exceptions has not been as well trained in the postmortem room, which is necessary to correctly correlate radiographic appearances with the hard facts of morbid anatomy. (4) As a consequence, forms of thoracic disease which the postmortem room shows to be rare or nonexistent have become common. He calls attention to the unusual frequency of peribronchial tuberculosis and the frequent mention of pulmonary fibrosis in America since the advent of radiography.

The volume should be of distinct value to those interested in tuberculosis, while the author's criticism of the clinician's attitude toward diagnosing incipient tuberculosis should be read with profit by the latter group.

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*Aids to Practical Pathology**

AS STATED in the preface, this book deals practically entirely with clinical pathology. It is a small reference hand book for laboratory use. The first few pages are devoted to an index alphabetically arranged. Some of the more recent observations, such as those on the various types of pneumococcus and streptococcus, the toxin of B. botulinus, etc., are not incorporated. Considerable space is given to urinalytic methods which are rarely used, while perhaps too little space is devoted to certain other procedures as the examination of feces for parasitic ova, etc. Chemical and serologic examination of the blood is very well condensed in this volume. The book is intended primarily for students, and in view of this the inclusion of unusual examinations is readily understood.

Rejuvenation Without Operation

A BROCHURE from the "American Institute of Radiendocrinology," based on the radiation technic of Eugen Steinach of Vienna. By Herman H. Rubin, M.D., Director of the American Institute of Radiendocrinology. Cloth. Pp. 64. Published by Medical Science Publishing Co., New York, 1923.

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*Aids in Practical Pathology: By F. W. W. Griffin, M.A., M.D., B.C., (Cantab) M.R.C.S. (Eng.) L.R.C.P. (London, Asst. Pathologist, Virol Pathological Research Labs.); Late Capt. R.A.M. Pathologist; Formerly Tuberculosis Officer, County of Survey, etc, and W. F. M. Thompson, Chief Technical Assistant, Virol Pathological Research Labs. Late Asst. in the Labs. of the Brompton Hosp. for Consumption; the Surrey County Council; the Royal Herbert Hosp., Woolwich, the Lister Institute, etc. Cloth. Price $1.50. P. 246. William Wood & Company. 1923.

The American Society of Clinical Pathologists

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The American Society of Clinical Pathologists

It was with an unusual sense of satisfaction that one viewed the gathering of workers in the field of scientific medicine on the third annual convention of the American Society of Clinical Pathologists.

This Society was organized for the purpose of stimulating the interest and furthering the development of scientific methods as applied to the diagnosis and treatment of disease. The germ of its inception sprang from a conversation between two clinical pathologists of Denver, Colorado, in the spring of 1921, when it was decided to call together those physicians engaged in the special field of clinical pathology in that city—some nine in all—for the purpose of arranging a symposium on the subject of "blood chemistry" before the Medical Society of the City and County of Denver. This meeting was held April 5, 1921, and proved so successful that a permanent organization was established in Denver with the purpose of convening at monthly intervals.

At the first session it was determined that the clinical pathologists in other cities of the State of Colorado be invited to participate. This eventuated in the formation of the Colorado Society of Clinical Pathologists. At the second meeting of this new state Society, the question of the formation of a national organization was discussed and the secretary was instructed to forward appropriate questionnaires to all physicians listed as clinical pathologists in the directory of the American Medical Association, in order to determine the interest which might be enlisted in the development of this organization.

Out of three hundred fifty of such communications, replies were received from one hundred fifty-two who expressed their willingness to co-operate.

Through the good offices of Dr. George Ives, arrangements were made to meet in the auditorium of the Missouri Baptist Sanitarium in St. Louis, at the time of the meeting of the American Medical Association in May, 1922. At this session of two days, permanent officers were chosen and the constitution and by-laws were adopted. The success of the second annual convention held in San Francisco last year established the permanency of the organization, which, as was apparent from that gathering, now assumes a place as one of the foremost scientific bodies of the country.

Since the objects of this Society are "to promote the practice of scientific medicine by a wider application of clinical laboratory methods to the diagnosis of disease; to stimulate original research in all branches of clinical laboratory work; to establish from time to time uniform standards for the performance of various laboratory examinations; to elevate the scientific and professional status of those specializing in this branch of medicine; to encourage a closer cooperation between the practitioner and the clinical pathologist," it may be seen that there is in the foreground a broad field of usefulness. Indeed, it must be apparent that the Society has accomplished a great deal during the brief period of its

existence. The blatant advertising of commercial laboratories in medical journals, has been greatly modified. The status of the clinical pathologist is being gradually raised from a mere technician to that of a consultant in medicine on a plane with the surgeon and internist. The American College of Surgeons, recognizing in this organization its logical collaborator in establishing adequate laboratory service in modern hospitals, very graciously solicited our views before their last convention and some of the suggestions presented have since been adopted.

One of our foremost aims, it would seem, should be to make the field of clinical pathology attractive to young physicians emerging from the medical colleges. This can be accomplished through persistent effort to elevate both the scientific and economic status of this branch of medicine.

The growth of the Society is no longer a matter for concern. The adoption of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE with its large circulation, as the official organ, furnishes an ideal medium of expression, and it may be safely predicted that practically all eligible clinical pathologists of the country will recognize the expediency of affiliation. The membership is now two hundred and fifty, and I anticipate an increase of possibly double this amount during the coming year.

The Society has been particularly fortunate in the selection of its presiding officers. Dr. Hillkowitz, whose high ideals piloted our bark safely over the shoals of our first annual voyage, has been an indefatigable worker from the beginning.

Dr. MacCarty has likewise taken a most active part in the organization and conduct of the Society from its birth, and it was through his efforts that the third annual convention proved so attractive.

And now the convention has chosen for its leader one of the most outstanding men in our specialty—Dr. John A. Kolmer, who expressed his full appreciation of the responsibilities of his office and under whose leadership the society will go forward to the accomplishment of ideals that will still further raise the status of our profession.

With regard to the future aims and objects, it would seem appropriate to refer to the words of our former President in a recent editorial in the Journal of Laboratory and Clinical Medicine, when he states "pick out the practical diagnostic and therapeutic facts and methods from those of pure scientific interest; standardize these facts and methods; set a standard educational requirement for laboratory consultants to obviate the rapid increase of false claims of technicians who frequently conduct commercial laboratories, the value of which many physicians are unable to determine; maintain laboratory sciences within the realm of the profession of medicine and not allow them to become merely commercial adjuncts; stimulate the interest of the general profession in the real value of the laboratory in the practice of medicine and surgery; and, assist in the standardization of hospitals."

With these principles before us and a firm and steadfast determination to adhere to and be guided by them, the value and usefulness of this Society will be realized as inseparable from the ideals to which modern scientific medicine aspires.

WARD BURDICK.

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Let your phraseology express one meaning and one only. Be clear.[2]

Manuscript.—Manuscripts should be typewritten, with wide margins, and double spaced, on one side of paper 8½ by 11 inches in size. The original copy should be sent to the "Journal" and the carbon copy retained by the author. Number the leaves consecutively, beginning with the title page. Put your name and address on the manuscript.

Illustrations.—Illustrations should be clear, preferably pen-and-ink drawings. Of photographs send a good print rather than a negative. Have lettering parallel to the bottom and top margins, and of sufficient size to be clear if cut is to be reduced. Tracings should be in black-and-white; avoid colors. Write your name on back of each picture; number them in one series (Fig. 1, etc.) to the end, and indicate in margin of the manuscript about where each is to be printed. See that the text references and "figures" correspond. Legends for illustrations should be written on a separate sheet.[3]

Bibliographic References.—Give only references actually consulted. If an article is known only through an abstract give reference to the abstract in addition to that of the source. References are printed to be of help in further reading; therefore they must be complete, concise, and correct. Follow the style of the "Index Medicus" and "Index-Catalog of the Library of the Surgeon-General's Office." Be conservative in the use of abbreviations.[4]

Arrangement.—As authors are quoted in the text give each a number in the order of citation, and number the bibliographic reference with the same number. Arrange the references in a list at the end of the article in the order of the numbers (see below), or arrange items in alphabetical order according to last names of authors, and distinguish between articles by the same author by the use of the date after his name in the text.

Foot-notes.—Where an author wishes to use foot-notes at bottom of each page instead of the bibliography at end of article, the foot-notes should be written in the text, but separated from it by horizontal lines above and below, or *better*, place them at bottom of each page. Use figures to indicate these foot-notes, and number consecutively (1, 2, 3, etc.) throughout the article. If in addition to the bibliography mentioned above it is desired to use foot-notes on certain pages, these can be indicated by an asterisk (*).

Final Reading.—Let some one other than the author read the manuscript with these directions in mind.

Shipment.—Send manuscript flat, postage paid, to the editor, Dr. Warren T. Vaughan, Medical Arts Bldg., Richmond, Va.

Proof-reading.—Read carefully, with special attention to spelling of names and bibliographic data. Make corrections *in the margin* only with lines drawn from the revision to the point of change in the text. Answer queries in the proof by making correction or crossing out the query. Verify your references from the sources, not from your carbon copy.

References. (Read these.)

[1]Billings, J. S.: Our Medical Literature, Trans. VII Intern. Med. Congress, Lond., 1881, i, 54-70.

[2]Mayer, Emil: Medical Literature and its Preparation, Med. Record, N. Y., 1915, lxxxvii, 1019-1021.

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McCrae, Thomas: The Use of Words, Jour. A. M. A., Chic., 1915, lxv, 135-139.

[3]Suggestions to Medical Authors, issued by the A. M. A. Press, Chic., A. M. A., [1914 (?)].

[4]Place, F.: Bibliographic Style in Medical Literature, Med. Record, N. Y., 1913, lxxxiii, 157-160.

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